SANDIA REPORT

An Ecological Study of Arsenic-Related Bladder Cancer in U.S. Counties: Effects of Reference Populations and Confounders on the Calculated Risks

Malcolm D. Siegel

Sandia National Laboratories

Issued by Sandia National Laboratories, operated for the United States Department of Energy by Sandia Corporation.

An Ecological Study of Arsenic-Related Bladder Cancer in U.S. Counties: Effects of Reference Populations and Confounders on the Calculated Risks

Malcolm D. Siegel
Geochemistry Department
Sandia National Laboratories
Albuquerque, NM 87185

January 2006

Abstract

The effects of the choice of reference populations on standardized mortality ratios and standardized incidence ratios (SMRs and SIRs) are demonstrated in an ecological study of the relationship between bladder cancer and exposure to arsenic in drinking water. Data from the Surveillance, Epidemiology and End Results (SEER) and the Center for Disease Control (CDC) WONDER databases were used to calculate county-level SMRs and SIRs for several populations of white males for the time period 1982 - 1998. Standardized rates were calculated for exposed populations in 36 United States (US) counties that consume water with arsenic concentrations higher than the new US Environmental Protection Agency (EPA) drinking water standard (10 ppb). When bladder cancer mortality rates in the exposed counties were compared to rates in a US standard population, the SMR was 0.79 (95% CI = 0.73 - 0.87). When rates in the exposed populations were compared to rates for a reference population drawn from 105 adjacent unexposed counties, the SMR was not significantly different from 1.0 (SMR = 0.93; 95% CI = 0.85 - 1.02). A similar pattern was observed when exposed counties and unexposed counties were limited to New Mexico and Utah.

Standardized incidence ratios also showed dependence on the choice of the reference population. For exposed populations in New Mexico, the SIR was less than 1.0 (SIR = 0.86; 95% CI =0.82 - 0.91) when the US standard population was used but was significantly greater than 1.0 when the contiguous unexposed counties were used as the reference population (SIR =1.20; 95% CI = 1.13 - 1.26). For the two exposed counties in Utah, the SIR was also significantly less than 1.0 (SIR = 0.65; 95% CI =0.46 - 0.89) when the reference population was based on the US standard population, and was higher but not statistically significant when the reference population was based on local unexposed counties (SIR = 0.97; 95% CI = 0.68 - 1.33). Although the use of local reference populations might decrease uncontrolled confounding and bias, the calculated risks for bladder cancer are likely subject to residual errors. These include potential confounding due to smoking prevalence and prior occupational exposures, migration, and misclassification bias. This study helps to illustrate potential limitations in ecological studies that use SMR and SIR to determine the relationships between cancer risks and arsenic exposure in the United States.

Acknowledgments

Table of Contents

Deleted: 9
Deleted: 9
Deleted: 11
Deleted: 11
Deleted: 11
Deleted: 11
Deleted: 14
Deleted: 14
Deleted: 14
Deleted: 14
Deleted: 14
Deleted: 14
Deleted: 16
Deleted: 16
Deleted: 18
Deleted: 18
Deleted: 20
Deleted: 20
Deleted: 21
Deleted: 21
Deleted: 22
Deleted: 22
Deleted: 22
Deleted: 22
Deleted: 23
Deleted: 23
Deleted: 24
Deleted: 24
Deleted: 27
Deleted: 27
Deleted: 29
Deleted: 29
Deleted: 30
Deleted: 30

3

Figures

Tables

Deleted: 13
Deleted: 13
Deleted: 17
Deleted: 17
Deleted: 18
Deleted: 18
Deleted: 9
Deleted: 9
Deleted: 15
Deleted: 15
Deleted: 15
Deleted: 15
Deleted: 16
Deleted: 16
Deleted: 19
Deleted: 19
Deleted: 19
Deleted: 19
Deleted: 19
Deleted: 19
Deleted: 20
Deleted: 20
Deleted: 20
Deleted: 20
Deleted: 24
Deleted: 24
Deleted: 28
Deleted: 28

Acronyms

AOED	Arsenic Occurrence and Exposure Database
AwwaRF	American Water Works Association Research Foundation
BFD	blackfoot disease
BRFSS	Behavioral Risk Factor Surveillance System
DMA	dimethylarsinic acid
EPA	Environmental Protection Agency
MH	Mantel Haenszel
LDS	Latter-Day Saints
MCL	maximum concentration level
MMA	monomethylarsonic acid
NAS	National Academy of Sciences
NRC	National Research Council
ppb	parts per billion
PRR	prevalence risk ratio
ROS	reactive oxygen species
RR	relative risk
SAM	S-adenosyl methionine
SEER	Surveillance, Epidemiology and End Results
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SWDIS	Safe Water Drinking Information System
TCC	transitional cell carcinoma
US	United States

This page intentionally left blank.

Introduction

On October 31, 2001, the United States (US) Environmental Protection Agency (EPA) finalized a proposed arsenic standard for drinking water that lowered the maximum concentration level (MCL) from 50 parts per billion (ppb) to 10 ppb [1]. The new standard was set to prevent approximately 28 deaths per year from lung cancer and bladder cancer. It was based in part on reviews of the scientific literature carried out by the National Research Council (NRC) of the National Academy of Sciences (NAS) at the request of the US EPA [2]. The NAS concluded that the risks were high enough to justify reduction of the existing arsenic drinking water MCL from 50 ppb to a level of 10 ppb or lower. The NAS report based its findings primarily on studies carried out for populations in Taiwan, Bangladesh, and Latin America that consume water with arsenic concentrations substantially higher than 50 ppb.

The subcommittee placed less emphasis on the few studies of populations in the United States exposed to the lower arsenic concentrations common in many drinking water systems that would be treated under the new standard. These studies have found little evidence of increased risks for bladder and lung cancer [3,4] at arsenic concentrations between 50 ppb – 100 ppb. However, the NAS subcommittee felt that such studies could not be used as a basis for quantitative risk assessment [2]. For example, the NAS felt that selected reference population used in recent study[3] of populations in Utah weakened the validity of its findings of no excess bladder or lung cancer risks. A more comprehensive epidemiological study of the effect of the choice of reference population on calculations of the risk ratios is clearly warranted by the importance of this issue for public health. In this study, an ecological study design was used to calculate standardized incidence and mortality ratios for bladder cancer in counties where arsenic concentrations in drinking water exceed the new US EPA MCL of 10 ppb. The sensitivity of the risk estimates to the choice of the reference population was examined by comparing the standardized rates obtained using US standard disease rates, state rates and rates in adjacent counties.

Literature Review

Bladder cancer is the sixth most common form of cancer in the United States [5]. It is the fourth most common cancer among men and the ninth most common among women [6]. The American Cancer Society estimated that the incidence and mortality for the year 2000 in the United States were 53,200 cases and 12,200 deaths, respectively [5]. The world-wide incidence of bladder cancer in 1996 was 300,000 cases [7]. Data from the Surveillance, Epidemiology and End Results (SEER) program show that 1) bladder cancer is more 4 times more common among men than women; 2) the incidence rate among whites is twice that of blacks in the US; 3) mortality rates for Hispanics and Asians are about ½ those for whites and blacks, and 4) the risk of bladder cancer increases with age. Rates for people 70 years old and older are 2-3 times higher than those aged 55-69 years and 15-20 times higher than those aged 30-54 years [8, 9]. The natural history of the disease is described in more detail in Attachment A.

Bladder cancer incidence rates are highest in industrialized countries (United States, Canada, France, Denmark, Italy and Spain). From 1993 – 1997, the mortality rate of bladder cancer in New Mexico was the lowest of any state (2.4 per 100,000); Maine had the highest

mortality rate (4.3 per 100,000) [9]. Incidence and mortality rates of bladder cancer have been related to many lifestyle, occupational, and environmental factors. A strong relationship between bladder cancer and smoking has been established by a large number of epidemiological studies [10,11]. Bladder cancer is considered to be the model of chemical carcinogenesis [12]. Occupational exposures might account for as much as 25% of all bladder cancers [13]. Toxicological studies in the early to mid 20th century established the role of aromatic amines (arylamines) in causing cancer. The use of these compounds in the paint, pesticide, pharmaceutical and chemical industries is associated with increased incidence of bladder cancer [14,15,16]. The presence of these compounds in cigarette smoke might be responsible for the strong association between bladder cancer and smoking.

Consumption of arsenic in drinking water is the main focus of this work, however, other exposure pathways are important. Anthropogenic sources of arsenic include mining, smelting, combustion of fossil fuel and agricultural activities. Arsenate (As(V)) has been used in glass making, pigments and pesticides, desiccants, wood preservatives, sheep and cattle dip and for weed control. Other possible arsenic human exposure modes include consumption of vegetables grown in arsenic-contaminated soils, consumption of shellfish, and direct ingestion of As-contaminated soils.

Arsenic and Mechanisms of Bladder Carcinogenesis

In a review of recent research, Kitchin [17] described nine different possible modes of arsenic carcinogenesis. These include induced chromosomal abnormalities, oxidative stress, altered DNA repair, altered DNA methylation patterns, altered growth factors, enhanced cell proliferation, promotion/progression, gene amplification, and suppression of p53. As(III) forms strong bonds with sulfur, therefore, arsenic binding to the sulfhydryl groups of cysteine is important for a number of protein and enzyme systems. It is estimated that As(III) can bind to and inhibit the activity of at least 200 proteins [2].

The majority of arsenic that is eliminated in urine by humans is in the form of methylated arsenicals. Typically, 10-30% of the arsenic is in inorganic forms, 10-20% is in the form of monomethylarsonic acid (MMA) and 55-75% is excreted as dimethylarsinic acid (DMA) [18]. Both methylated As(III) and As(V) species are found in humans; the DMA(V) species is predominant. Methylation of arsenic occurs primarily but not exclusively in the liver and produces a number of As(III) and As(V) intermediates. The process includes a number of oxidation, reduction and methylation steps. These involve the activities of S-adenosyl methionine (SAM) and Glutathione (L-γ-glutamyl-L-cysteinylglycine or GSH).

Recent studies indicate that the methylated forms of arsenic are both toxic and directly carcinogenic. Both DMA(III) and MMA(III) can cause enzyme inhibition, cell toxicity and genotoxicity. Kitchin [17] reviews studies that demonstrate that DMA acts as either as a promoter or complete carcinogen for bladder cancer. Bladder cancer may result from arsenic exposure because this organ contains relatively high concentration of DMA and MMA in the lumen. Methylated forms of arsenic can produce oxidative stress by creating reactive oxygen species (ROS) that attack DNA. The most important mechanisms for bladder cancer are described in more detail in Attachment A.

Bladder Cancer and Exposure to Arsenic in Drinking Water

Drinking water is a major source of exposure to arsenic. Naturally occurring arsenic is associated with aquifers that contain rocks from volcanic sources. It is strongly enriched in silicic volcanics, derived volcanoclastic sediments, hydrothermal systems, and rocks affected by potassium metasomatism, a low-temperature alteration process common in closed hydrographic basins in arid climates. Arsenic concentrations range from 1.1 ppb to 6000 ppb in the 7000 water samples collected in the Western United States and described in a study by Welch et al.[19]

A large number of epidemiological studies examining the relative risks for health effects associated with arsenic exposure in drinking water have been carried in the last three decades. The most recent comprehensive review of these studies was carried out by the Subcommittee to Update the 1999 Arsenic in Drinking Water Report, Committee on Toxicology of the National Research Council [2]. Table 1 summarizes several of the major ecological studies and indicates the arsenic exposures that have been associated with statistically significant increased risk for bladder cancer. It is noteworthy that in nearly all of the studies reviewed, significantly elevated risks were found only for populations who consume drinking water with arsenic concentrations considerably higher than 50 pbb.

Table 1. Previous Ecological Studies Showing Associations between Bladder Cancer and Arsenic Exposure Among Males

Study	Cases	SMR	95% CI	Concentration (ppb)	Comment
Tsai et al. [22]	312	8.92	7.96 – 9.96	250 – 1140 (median = 780 ppb)	Ecological study in black-foot disease endemic area; SMR for regional reference population.
Chen et al.[20]	167	11.00	9.33 - 12.67	Two groups: Artesian wells: 350 – 1140 (median = 780) and shallow wells: 0 – 300 (median = 40 ppb)	Ecological study in black-foot disease endemic area; statistical significance not given for shallow wells
Hopenhayn-Rich et al.[26]	113	0.80	0.7 – 1.0	<40 ppb, low skin cancer	Reference population was all Argentina; unclear method of exposure classification for counties based partly on prevalence of skin cancer.
	93	1.42	1.1 – 1.17	scattered high (>100 ppb)	
	131	2.14	1.8 – 2.5	40 – 433; average= 178 ppb	
Smith et al.[25]	93	6.0	4.8 – 7.4	Average ranged from 43 – 569 ppb over period 1950 – 1994; 110-870 ppb for 2/3 of population in 1955- 1979.	Claim data from pulmonary disease show that smoking was not a contributor to increased risk.

Several ecological studies of arsenic exposure and mortality from internal cancers in areas of Taiwan with high arsenic exposures have been carried out. Chen et al. [20] studied bladder cancer mortality rates for males over the period 1968-1983 in the southwest coastal region of

9

Taiwan where blackfoot disease (BFD) is endemic. They calculated an overall standardized mortality ratio (SMR) of 11.00 (95% CI = 9.33 – 12.67), irrespective of the depth of the drinking water source and BFD incidence, using Taiwan national rates for a reference population. They found a positive correlation between bladder cancer rates and BFD incidence rates and calculated higher SMRs for the populations using deep artesian wells compared to those using shallow wells for drinking water. The SMR for populations using shallow wells with lower arsenic concentrations (0 – 300 ppb; median = 40) was approximately 5, however, confidence limits were not reported.

Chen and Wang [21] carried out an ecological study for Taiwan as a whole and calculated age-adjusted bladder cancer mortality rates for 314 townships and precincts over the period 1976 - 1983. A direct age-adjustment was made using the 1976 world population as the standard population. They found a significant dose-response relation between bladder cancer mortality and the average arsenic concentrations of precinct or town wells. Reported arsenic concentrations in individual drinking water wells ranged from >50 ppb to >350 ppb, however the range for the average As concentrations in the each precinct or township was not reported. After adjusting for industrialization and urbanization, they found an increase of 3.9 ±0.5 bladder cancer deaths/100,000 person years for each 100 ppb increase in the average arsenic concentration using a population-time-weighted regression analysis.

Tsai et al. [22] calculated SMRs for bladder cancer for males in four townships of the BFD-endemic region of the southwest coast of Taiwan during the period 1974 -1994. Arsenic concentrations in water from the primary source of water (artesian wells) ranged from 250-1140 ppb (median = 780 ppb). When Taiwan national rates were used for the reference population, the SMR was 10.5 (95% CI = 9.37 – 11.73); when a local reference population was used, the SMR was 8.92 (95% CI = 7.96 – 9.96).

The relationship between bladder cancer incidence and arsenic exposures in Taiwan has also been studied. Guo et al. [23] examined incidences of specific types of urinary tract cancers in all of Taiwan using an ecological study design (total cases = 1341). They used the proportions of wells with specified levels of arsenic in each of 243 townships as an indicator of exposure during the period 1980 - 1987. Arsenic concentrations ranged from <50 ppb to >640 ppb. They found that only the proportion of wells with arsenic concentrations >640 ppb had a positive association with the incidence of transitional cell carcinoma (TCC) of the urinary bladder. For every 1% increase in this proportion, there was an increase of 0.57 bladder cancers per 100,000 person-years in males.

Chiou et al. [24] carried out a cohort study of the incidence of TCC from 1991–1994 in a population of 8,102 residents in an arseniasis-endemic area in NE Taiwan. Arsenic concentrations in drinking water ranged from 10 ppb to >100 ppb. Overall, the standardized incidence ratio (SIR) for bladder cancer (total cases = 10) was 1.96 (95% CI = 0.94 – 3.61) when the general population in Taiwan was used for the reference rates. The multivariate-adjusted relative risks of developing TCC were 1.9 (95% CI = 0.1 – 32.5), 8.2 (95% CI = 0.7 – 99.1), and 15.3 (95% CI = 1.7 – 139.9) for arsenic concentrations of 10.1 - 50.0, 50.1 –100.0 and >100.0 ppb respectively.

Ecological studies have been carried out by Smith and coworkers in several areas of South America where populations consume drinking water with high arsenic content[25,26]. These studies indicate statistically significant elevated risks for bladder cancer and several other

cancers in population groups exposed to arsenic concentrations ranging from <40 ppb to 870 ppb. Strongest evidence for an association between arsenic exposure and bladder cancer was found in groups with high (>100 ppb As) exposure. For example, the SMR for males over the period 1989–1993 was 6.0 (95% CI = 4.8 – 7.4) in a region in Northern Chile where the average arsenic concentration was 420 ppb. The Chilean national mortality rates in 1991 were used for the standard population and the arsenic exposure concentrations were the population-weighted averages for the major cities or towns [25].

Studies of populations in Utah were carried out by Bates et al. [27] and Lewis et al.[3] Bates et al. [27] conducted a case-control study using incident cases identified in the 1978 National Bladder Cancer Study.   The overall association between arsenic exposure and bladder cancer was not statistically significant. Dose-response trends were evident for smokers in exposure periods 30-39 years prior to diagnosis; associations with moderate significance (p = 0.1) were observed in only 2 of 30 subsets examined. Lewis et al. [3] conducted a cohort study of approximately 4,000 residents of Millard County, Utah. Cumulative arsenic exposures were calculated from median arsenic concentrations (14 – 166 ppb) in community drinking water and estimates of residence times of cohort members in the wards of the Church of Jesus Christ of Latter-Day Saints (LDS). SMRs were calculated for a number of cancer and non-cancer outcomes using the Utah state mortality rates for the reference population. The study failed to find any statistically significant increased risks for bladder cancer and most other cancers but calculated a significantly reduced SMR for cancers of the respiratory system.

The NAS report [2] criticized the metric used for exposure in the study by Lewis et al. [3] suggesting that the approach is likely to lead to underestimation and misclassification of exposure when the concentrations vary over the wide ranges observed in the Utah communities. In addition, the use of a metric that combines exposure intensity (i.e. median arsenic concentration) and duration (i.e. years of residence) can group people with very different exposure histories into the same exposure stratum. Finally, the NAS criticized the choice of reference population in that study and suggested that confounding might have affected the results. It is possible higher smoking prevalence (and higher associated cancer mortality rates) in the state reference population concealed a higher SMR for bladder cancer in the population exposed to arsenic (Millard County), which is primarily LDS and presumably has lower smoking prevalence because of religious beliefs.

Methods

Exposed and Unexposed Populations

Exposed populations were defined as white males living in counties in which the majority of the population consumed drinking water from regulated community wells with mean arsenic concentrations of 10 ppb or greater. It was assumed that the current or recent concentrations reported in the data sources listed below could be used to represent exposures prior to the health effects occurring from 1982-1998.

The arsenic concentrations in drinking water were estimated using a compilation by Frost[28] that was prepared for the American Water Works Association Research Foundation (AwwaRF). Databases from several sources were used to identity counties with exposed

populations[29,30,31,32,33]. The final list of 36 counties (Appendix I) was compiled by combining these data with additional information obtained through telephone interviews with staff from water utilities as described by Frost[28]. This list differs slightly from the one described in later publications[34,35].

Several criteria were used to classify counties as "exposed" for this study, including: 1) the mean arsenic concentration in drinking water for the county was 10 ppb or greater; 2) at least half of the population was served by a public water system; and 3) the dominant source of drinking water was groundwater and not surface water. The arsenic concentrations listed in Appendix I were obtained in a two-step process: 1) data from water utilities were used to calculate average values for each city within an exposed county and 2) a populated-weighted mean concentration for the county was calculated using the means for the cities and the city population data from state data or the US EPA Arsenic Occurrence and Exposure Database (AOED)[29]. More detailed descriptions of the selection process and the method used to calculate the average arsenic concentrations for the exposed counties are given by Frost et al. [28,34,35].

All of the "exposed" counties were included in the analysis of the bladder cancer mortality data from the CDC WONDER database. Figure 1 shows the distribution of exposure levels in the exposed population from 1982 - 1998. About 70% of the exposure occurred in the range 10 – 20 ppb; less than 2% occurred at arsenic concentrations above 50 ppb, the old EPA drinking water standard for arsenic. The population-weighted mean arsenic exposure level was 19.6 ppb.

Bernalillo County, New Mexico, was the only large urban county included in this study. This county accounted for approximately 30% of the entire exposed person-years of the study. Separate analyses were carried out in which the population of Bernalillo County was excluded in order to evaluate the sensitivity of the results to the characteristics of this single large county. Five exposed populations of white males were defined for this study: 1) the "national exposed population" comprised of the 36 exposed counties listed in Appendix I, 2) the "national exposed population" without Bernalillo County, New Mexico, 3) exposed counties from New Mexico, 4) the NM exposed population without Bernalillo County, and 5) exposed counties from Utah. Incidence data were available only from counties in New Mexico and Utah, which are part of the SEER database[36]. Therefore, comparisons of incidence and mortality could be made only for these two states.
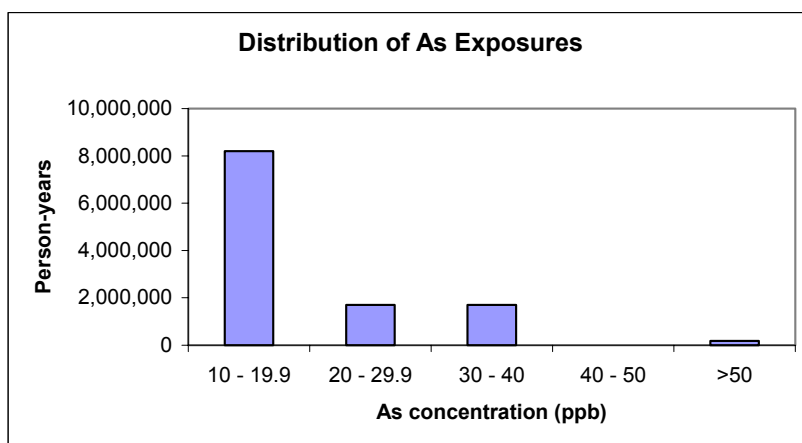
Figure 1. Distribution of arsenic exposures by person-years in 34 counties in which arsenic concentration in drinking water was 10 ppb or greater.

Three types of reference populations of white males from 1982 – 1998 were used for calculations of standardized rates: 1) the US population, 2) individual state populations (Utah and New Mexico), and 3) local reference populations comprised of counties adjacent to the exposed counties described previously ("adjacent unexposed counties"). The adjacent unexposed counties were identified from maps on the *USA Counties 1998* website of Oregon State University – Information Services[37]. Adjacent unexposed counties were usually defined as counties that shared a common border with the exposed counties; in some cases, these included counties in neighboring states. However, the criterion for inclusion as an adjacent county was fairly loose; in a few cases, a county was considered adjacent even if it did not share a border but was separated from the exposed county by a small (<20 miles) section of another county. The initial list of adjacent counties was then screened to exclude counties with relatively large urban populations or industrial centers, and counties in which locally high concentrations of arsenic were reported in any of the databases cited above [30-33,38]. A total of 105 unexposed counties were identified in this manner and are listed in Appendix II. For calculations involving only exposed counties in New Mexico or Utah, only unexposed adjacent counties within the state were used for the local reference populations.

Demographic and socio-economic data for exposed and unexposed counties were obtained from the 1990 U.S. Census[39]. These included ethnicity, education, urbanization, occupation (that might lead to arsenic exposure) and income. Aggregate values for each population were obtained by summing the variable-specific population counts over all the counties in that population. In some cases, county lines have been redrawn and some of those in the 1990 Census may not correspond to those used by the CDC and SEER over the period 1982–1998 as described below.

13

Calculation of Standardized Mortality and Incidence Ratios

Mortality data for bladder cancer for the period 1982 to 1998 for white males were obtained from the CDC WONDER database[40] for Revision 9 of the International Classification for Diseases (ICD-9) codes 188 – 188.9[41]. Standardized mortality ratios (SMR) for white males were calculated for the 36 counties with arsenic concentrations higher than 10 pbb, i.e., " the national exposed population" (Appendix I), as well as the subsets of the exposed counties described above. Incidence data for the corresponding SEER topographies were obtained from the SEERSTAT program[36] to calculate SIRs for exposed population in New Mexico and Utah. Reference populations for SMRs and SIRs were based on US national rates, rates for Utah and New Mexico, and the aggregated rates for the unexposed counties as described above.

The procedure that was used to calculate SIRs and SMRs in described in detail in Appendix III. Total observed numbers of bladder cancer deaths and incident cases for 10-year age intervals were obtained from the CDC WONDER and SEER databases, respectively, for the period 1982 – 1998 for the counties with high arsenic concentrations (>10 ppb). Reference mortality and incidence rates were obtained from the total observed age-specific numbers of bladder cancer deaths and incident cases and the age distributions in the unexposed counties.

Results

Characteristics of Exposed and Unexposed Populations Based on 1990 Census

Tables 2 to 4 summarize several characteristics of the exposed and unexposed counties defined in the previous section. As discussed later, the attributes listed may be confounders in the relationship between exposure to arsenic in drinking water and bladder cancer. Table 2 shows that based on the 1990 Census, the population drawn from all 36 exposed counties in the US, was less urban, more Hispanic and had a slightly lower percent employed in occupations associated with exposure to arsenic, compared to the populations drawn from adjacent unexposed counties. When Bernalillo County, NM is removed from the exposed population, the resulting exposed population is more similar to the unexposed population in all categories except income.

The exposed population in New Mexico (Table 3) was more urban, more Hispanic, had a lower proportion of natives, had a higher percentage of Hispanics among the white male population between the ages of 25 and 85 (38% vs. 33%), and had a higher income than the unexposed population. When Bernalillo County is removed from the exposed population, the remaining exposed population is more similar to the unexposed population in degree of urbanization, percent native born, potential occupational exposures and income. The populations remain dissimilar in income and are more dissimilar in ethnicity. The exposed population now has a higher proportion of natives compared to the unexposed population. The exposed and unexposed populations from Utah (Table 4) were very similar; both were dominantly rural, less Hispanic and had higher levels of college education and income than the populations in Tables 2 and 3.

14

Table 2. Summary of Data from 1990 Census of Population Comprised of All Exposed and Adjacent Unexposed Counties in US in this Study.

	Exposed	**Unexposed**	**Exposed w/o Bernalillo Co.**
Total population	1,561,994	2,246,414	1,081,417
Urban	56.8%	75.2%	66.1%
Hispanic	25.5%	18.2%	20.4%
Non-Hispanic white	66.2%	70.7%	72.8%
Native born	54.7%	59.3%	57.7%
High school	21.0%	21.8%	21.0%
Bachelor's degree	8.2%	6.0%	7.1%
Potential occupational exposures	8.2%	9.6%	8.4%
Pop.-weighted median household income	$26,932	$26,732	$24,105

Table 3. Summary of Data from 1990 Census of Population Comprised of Exposed and Adjacent Unexposed Counties from New Mexico in this Study.

	Exposed	**Unexposed**	**Exposed w/o Bernalillo Co.**
Total population	638,260	324,557	157,683
Urban	86.6%	49.0%	59.0%
Hispanic	39.1%	26.6%	45.8%
Non-Hispanic white	52.0%	46.4%	40.1%
Hispanic white males 20-85 yrs	38.1%	33.4%	45.3%
Native born	51.7%	52.0%	63.5%
High school	21.4%	38.1%	22.7%
Bachelor's degree	9.4%	5.2%	5.6%
Potential occupational exposures	5.4%	5.0%	5.7%
Pop.-weighted median household income	$26,644	$20,453	$24,398

Table 4. Summary of Data from 1990 Census of Population Comprised of Exposed and Adjacent Unexposed Counties from Utah in this Study.

	Exposed	Unexposed
Total population	26,851	87,980
Urban	27.8%	34.6%
Hispanic	2.1%	2.8%
Non-Hispanic white	96.7%	92.7%
Hispanic white males 20-85 yrs	2.0%	2.5%
Native born	65.5%	77.6%
High school	16.9%	20.9%
Bachelor's degree	11.6%	5.2%
Potential occupational exposures	7.0%	9.1%
Pop.-weighted median household income	$32,374	$24,127

Descriptive Epidemiology of Bladder Cancer (1982-1998)

Data from the CDC WONDER Database and SEER were used to determine whether the bladder cancer mortality rates and incidence rates for white males, respectively, were relatively constant, varied synchronously, or varied randomly in the United States and in the specific states (New Mexico, Utah) over the time period studied in this analysis. The annual US age-adjusted mortality rates and incidence rates for the period 1982-1998 are shown in Figure 2. Annual incidence rates in the US ranged from approximately 47 to 52 cases per 100,000 person-years; annual mortality rates ranged from approximately 8 to 9 deaths per 100,000. In New Mexico and Utah, mortality rates ranged from approximately 4 to 8 deaths per 100,000. There are no apparent temporal trends in the incidence and mortality rates, the relative uniformity of the rates means that relative risks calculated from mortality data and incidence data should be comparable. In addition, these data suggest that the calculated SMRs and SIRs should not be an artifact of the specific time interval chosen for this study.

16

US Age-Adjusted Bladder Cancer Rates for White Males
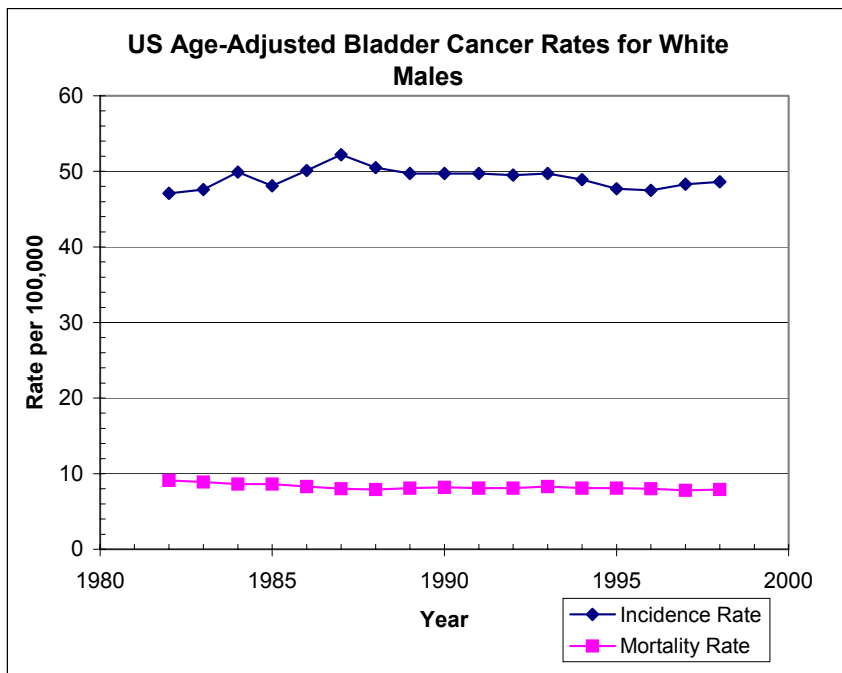
Incidence rates are from SEERSTAT [36] and are adjusted to 1990 US Standard population. Mortality rates are from CDC WONDER Database [40] adjusted to the 2000 US Standard population. Rates in person-years are for all bladder cancer stages.

Figure 2. Age-adjusted incidence and mortality bladder cancer rates for white males 1982-1998.

Age-adjusted mortality rates over the time period 1982–1998 for the 36 counties with high (>10 ppb) arsenic levels are summarized in Appendix I. Age-specific mortality and incidence rates for 1982–1998 are plotted in Figure 3. The sharp increase in incidence rates in white males older than 45-54 years demonstrates the importance of age adjustment of the rates for this analysis.

17

US Age-specific Incidence and Mortality Rates
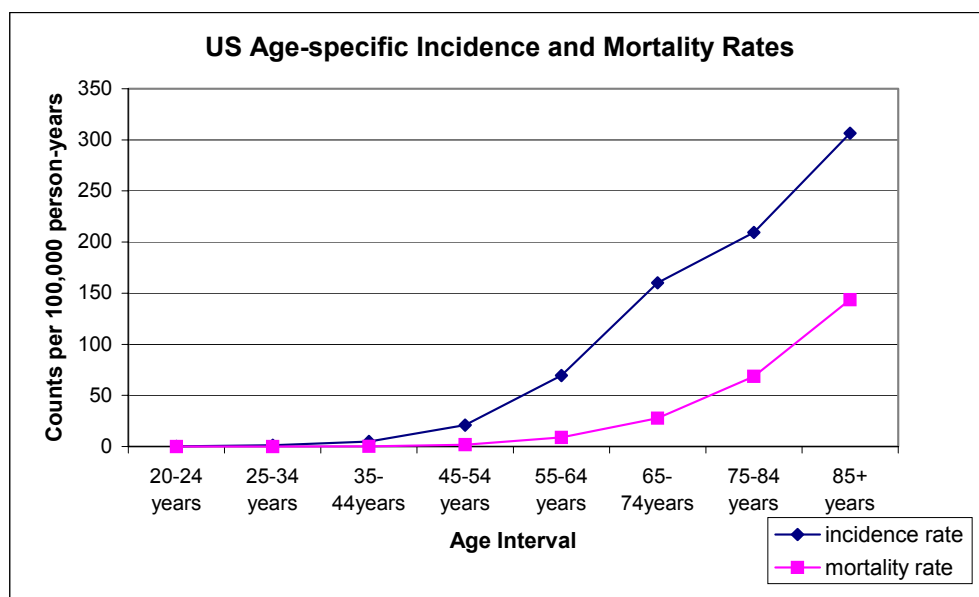
Incidence rate data are from the SEER 9 August 2000 submission[36] and include all registries and all bladder cancer stages. Mortality rate data are from the CDC WONDER Database[40].

Figure 3. Age-specific bladder cancer incidence and mortality rates for white males for the period 1982-1998.

Standardized Mortality Ratios

Table 5 shows the results of the SMR analysis for the 36 exposed counties and 105 unexposed counties listed in Appendices I and II, respectively. Separate analyses were carried out to evaluate the effect of the relatively large population of Bernalillo County on the calculated effects. These SMRs are statistically less than 1.0 whether the reference rates are taken from the 1982 - 1998 US Population or from the population of adjacent unexposed counties. The results are similar when Bernalillo County is removed from the list of exposed counties.

Standardized mortality ratios and 95% confidence intervals for exposed counties in New Mexico using different reference populations are shown in Table 6. Again, separate analyses were carried out to evaluate the effect of the large population of Bernalillo County on the calculated effects. If the US standard rates are used for the reference population, the SMRs are less than 1.0 at the 5% significance level, with or without Bernalillo Country. These results are not unexpected given the previous observations that the age-adjusted state mortality rate for New Mexico is among the lowest in the United States[9]. When the state age-specific mortality rates are used for the reference population, the SMR increases to 1.05 (95% CI: 0.91 –1.21). When the populations from the adjacent unexposed counties within New Mexico are used for reference rates, the SMR is similar (1.08; 95%CI: 0.94 – 1.24). The results do not change appreciably if Bernalillo County is excluded from the exposed populations.

18

Table 5. Standardized Mortality Ratios for 34 Exposed Counties* and 105 Adjacent Unexposed Counties* in 11 States.

Reference	Exposed	Observed	Expected	SMR	95% CI
US rates	Exposed counties	548	691	0.79	0.73 – 0.87
US rates	Exposed counties w/out Bernalillo County	405	499	0.81	0.74 – 0.90
US rates	Unexposed counties	1070	1264	0.85	0.80 – 0.90
Unexposed counties	Exposed counties	548	588	0.93	0.85 – 1.02
Unexposed counties	Exposed counties w/out Bernalillo County	405	424	0.95	0.86 – 1.05

*Exposed counties are listed in Appendix I; unexposed counties are listed in Appendix II

Table 6. Standardized Mortality Ratios for New Mexico.

Reference	Exposed	Observed	Expected	SMR	95% CI
US rates	Exposed NM counties	200	263	0.76	0.66 – 0.87
US rates	Exposed NM w/out Bernalillo County	57	70	0.81	0.61 – 1.05
US rates	Bernalillo County	143	192	0.74	0.63 – 0.88
NM state rates	Exposed NM counties	200	190	1.05	0.91 – 1.21
NM state rates	Exposed NM w/out Bernalillo County	57	51	1.12	0.85 – 1.45
Unexposed NM[*]	Exposed NM counties	200	185	1.08	0.94 – 1.24
Unexposed NM[*]	Exposed NM w/out Bernalillo County	57	49	1.16	0.88 – 1.50

*Unexposed counties include NM counties: Lincoln, McKinley, Mora, Otero, San Juan, San Miguel, Sierra and Taos.

Standardized mortality ratios and 95% confidence intervals for exposed counties in Utah using different reference populations are shown in Table 7. The SMRs increase in a manner similar to that described above for New Mexico; however, the 95% confidence intervals always include the value of 1.0 whether the reference population is based on US standard rates, Utah state mortality rates or the age-specific rates from the populations from the adjacent unexposed counties.

Table 7. Standardized Mortality Ratios for Utah.

Reference	Exposed	Observed	Expected	SMR	95% CI
US rates	Exposed Utah counties	9	11	0.82	0.37 – 1.55
Utah state rates	Exposed Utah counties	9	8	1.17	0.53 – 2.22
Utah unexposed counties*	Exposed Utah counties	9	5	1.72	0.78 – 3.26

*Unexposed Utah counties include Beaver, Duchesne, Juab, Morgan, Sanpete, Sevier, Uintah, and Wasatch.

Standardized Incidence Ratios

Tables 8 and 9 summarize the SIRs, 95% confidence intervals and the cumulative number of observed and expected bladder cancer cases for exposed populations in New Mexico and Utah from 1982-1998. The SIRs are all significantly lower than 1.0 when the US standard rates from the SEER database[36] are used for the reference population. The use of state populations for reference rates, however, produces qualitatively different results in some cases. When the New Mexico state rates are used for the reference population, the SIR for the exposed counties in New Mexico (Table 8), is greater than 1.0 at the 95% confidence level (SIR = 1.09; 95% CI: 1.03 - 1.15). However, the SIR is not statistically significant if Bernalillo County is removed from the exposed population (SIR = 1.05; 95% CI: 0.94 - 1.17). When the adjacent unexposed counties are used as the reference population, the SIRs for the exposed population of New Mexico are greater than 1.0 with or without Bernalillo County. For exposed counties in Utah (Table 9), a statistically significant elevated risk for bladder cancer incidence is not seen whether the reference rate is based on the state of Utah (SIR = 0.84; 95% CI: 0.59 - 1.15) or on the adjacent unexposed counties (SIR = 0.97; 95% CI: 0.68 - 1.33).

Table 8. Standardized Incidence Ratios for New Mexico.

Reference	Exposed	Observed	Expected	SIR	95% CI
US SEER	All New Mexico (NM)	2983	3751	0.80	0.77 – 0.82
US SEER	Exposed NM counties	1298	1504	0.86	0.82 – 0.91
US SEER	Exposed NM counties w/out Bernalillo county	323	386	0.84	0.75 – 0.93
NM state rate	Exposed NM counties	1298	1195	1.09	1.03 – 1.15
NM state rate	Exposed NM counties w/out Bernalillo County	323	307	1.05	0.94 – 1.17
Unexposed NM counties*	Exposed NM counties	1298	1086	1.20	1.13 – 1.26
Unexposed NM counties*	Exposed NM counties w/out Bernalillo County	323	279	1.16	1.04 – 1.29

*Unexposed NM counties include Lincoln, McKinley, Mora, Otero, San Juan, San Miguel, Sierra and Taos.

Table 9. Standardized Incidence Ratios for Utah.

Reference	Exposed	Observed	Expected	SIR	95% CI
US SEER	All Utah (UT)	2704	3497	0.77	0.74 – 0.80
US SEER	Exposed Utah counties	38	59	0.65	0.46 – 0.89
Utah state rates	Exposed Utah counties	38	45	0.84	0.59 – 1.15
Unexposed Utah counties*	Exposed Utah counties	38	39	0.97	0.68 – 1.33

*Unexposed Utah counties include Duchesne, Juab, Morgan, Sanpete, Sevier, Uintah, and Wasatch.

Discussion

This study examined the effect of the choice of the reference population on the standardized risks (SMRs and SIRs) calculated in an ecological study of bladder cancer and exposure to arsenic in drinking water. When exposed populations drawn from 36 counties in 11 states nationwide were compared to reference populations based on US standard rates, the SMR was significantly less than 1.0. When compared to reference populations drawn solely from adjacent unexposed counties, the SMR was higher but not statistically significant. A similar pattern was observed when mortality rates in exposed counties in New Mexico and Utah were compared to the rates in US standard population, the host state or reference populations drawn from adjacent unexposed counties within the state. None of the calculated SMRs were significantly greater than 1.0, regardless of the reference population that was used. Standardized incidence ratios also showed a dependence on the choice of reference population. For exposed populations in New Mexico, the SIR was significantly less than 1.0 when the rates from the US standard population were used as the reference but was significantly greater than unity when the adjacent unexposed counties were used as a reference population (SIR =1.20; 95% CI =1.13 - 1.26). For Utah, the SIR was also significantly less than 1.0 when the reference population was based on the US standard population. The SIRs were higher when the reference population was based on state rates or the adjacent unexposed counties but were not statistically significant.

The lack of a significant risk for bladder cancer mortality in exposed populations is consistent with the study of populations in Utah by Lewis et al. [3] as described above. The current study, however, differs from that of Lewis et al. [3] in the use of local populations for the reference rates. A few studies from other counties demonstrate an elevated mortality risk for bladder cancer at these relatively low (<50 ppb) arsenic concentrations in drinking water. Chen et al. [20] calculated a bladder cancer SMR of approximately 5 among males using shallow wells with low arsenic concentrations (0.0 – 300 ppb; median = 40 ppb) in southwest Taiwan. However, they do not provide any confidence limits. Kurttio *et al*. [42] found elevated risks for bladder cancer among smokers in Finland who had been exposed to arsenic concentrations above 0.5 ppb, three to nine years prior to cancer diagnosis. However, the high relative risks at such low water-borne arsenic exposures were unexpected (OR = 10.3; 95% CI: 1.16 – 92.6). The significance of the results is questionable due to poor exposure data; the main source of arsenic to the population was probably food rather than water.

Using the arsenic-associated health risk estimated by the National Research Council [2], Frost [34] calculated an expected maximum relative risk for arsenic-related bladder and lung cancer deaths for his list of exposed counties from 1950 to 1999 and adjusted for the effect of migration. For counties with arsenic concentrations higher than 20 ppb, the expected relative risk for bladder cancer was 1.16 (95% CI were not given); this is similar to that calculated in this study for arsenic exposures greater than 10 ppb in New Mexico.

A dependence of standardized bladder cancer risks on the choice of reference population was observed by Tsai et al. [22] for four townships of the BFD-endemic region of the southwest coast of Taiwan. However, the direction and magnitude of the effects were different from those observed in this study. The SMR for males was higher (10.5; 95% CI = 9.37 – 11.73) when

national rates were used for the reference population, compared to the rate calculated using a local reference population (8.92; 95% CI = 7.96 – 9.96).

Limitations

Ecological studies are characterized by the use of variables that describe the average characteristics of groups instead of individual-level measures. Such studies are most useful in generating new hypotheses and obtaining ecologic inferences about populations that cannot be obtained in individual-level measurements[43]. This study design is often criticized, however, because the relationships measured at the group level may not be the same as those measured at the individual level. The well-known "ecological fallacy" or ecological bias results from the false assumption that the inferences obtained from ecological studies apply either to individuals within the groups or to individuals across all groups. Piantadosi et al. [44] provide theoretical and empirical evidence that correlation and regression coefficients obtained from ecological studies inadequately represent both the corresponding across-group and the average within-group values. The sets of coefficients can differ in magnitude, significance and sign; they are dependent on the nature of the groupings and show no discernable qualitative consistency in these effects.

In this study, ecologic bias may result from misclassification and uncontrolled confounding. Both disease and exposure status could be misclassified in this study. Substantial migration could have occurred between the exposed and unexposed counties over the long time period examined in this study. Differential misclassification could lead to bias either away from or towards the null. Nondifferential misclassification often leads to potentially severe bias away from the null in ecologic studies. This is in contrast to the effect in individual-level studies where such misclassification typically leads to bias toward the null [43]. Uncontrolled confounding may be present given the lack of adequate data about the prevalence or identity of risk factors for bladder cancer in the populations at both the individual and aggregate level. However, confounding on the individual level might not produce ecological bias if it is ecologically unassociated with the exposure across groups [43].

Misclassification

Underestimation of bladder cancer mortality and incidence rates could lead to misclassification of disease status of counties in this study. As discussed by Frost [28], bladder cancer mortality may be underestimated because of the way deaths are coded on the death certificates (i.e. other, more immediate causes of death may be reported). Bladder cancer incidence may be underestimated due to the low effectiveness of screening programs[45] and the long latency of the disease. In addition, detection of bladder cancer may differ in the exposed and unexposed communities due to differences in access to health care, which are related to underlying socio-economic factors.

Exposure status of counties could be misclassified because the water quality data that was used do not accurately describe arsenic levels in the drinking water during the period of exposure. Frost [28,34] notes that there is great uncertainty in estimates of US populations obtaining drinking water with arsenic concentrations greater than 10 ppb. The problems include: 1) poor coverage of states in the EPA AOED data base (only 25 states are included); 2) lack of individual well production data for water utility systems with multiple source wells; 3) lack of any As concentration data in approximately 18% of the wells in the Safe Water Drinking

Information System (SWDIS) data base, which is the source of data for the EPA AOED; and 4) lack of adequate As concentration measurements at low levels (<20 ppb). For the period 1980 – 2000, 98.5% of groundwater systems in the state inventory (from SWDIS) in New Mexico and 97.9% in Utah had state compliance monitoring data for As; the nationwide compliance was 82% for all systems in the EPA AOED[34].

Recently published information suggests that the exposure status of some of the counties used in this study may be misclassified[34,35]. The list of exposed counties (Appendix I) was compiled in October, 2001 from data available at that time[28]. Frost [34] and Frost et al. [35] included a different updated list of counties with validated arsenic water concentrations greater than 10 ppb. The compilations for those studies did not consider the following counties as having As levels above 10 ppb: Rio Arriba, NM; Valencia NM; Elko, NV; Cleveland, OK; Midland, TX; Millard UT; and Twin Falls, ID.

Migration

It has been argued that in the United States, high migration rates and low environmental exposures make detection of arsenic health effects impossible[2]. Migration of individuals into or out of the source populations can produce bias because migrants and non-migrants can differ in exposure and in disease risk. Bladder cancer has a long latency period and temporal ambiguity exists because it is unclear if the exposure preceded the disease in a particular county. People could be exposed to arsenic in one county and then migrate to another county where the bladder cancer is detected. If migration were random across the two groups, then the bias would be toward the null in individual-level analyses. However, the effects of migration on ecologic studies are difficult to predict because nondifferential misclassification can bias the results away from the null[43].

For the populations in New Mexico, there is evidence that migration rates were different in the exposed and unexposed populations. Zhan[46] compiled population changes in counties in New Mexico from 1970 to 2000 using U.S. Bureau of Census Data. Results for the exposed and unexposed counties are shown in Table 10. It can be seen that the degree of population change over this time period is quite variable for different counties. Among the large exposed counties, Bernalillo and Sandoval Counties experienced changes of 76% and 414%, respectively. Among the unexposed counties, San Juan and Otero counties changed by 117% and 52%, respectively. It is also clear from the table that the exposed counties experienced greater changes in population than the unexposed counties (99% vs. 78%). When Bernalillo County is removed from the list of exposed counties, the difference in migration doubles (195% vs. 78%). The different migration rates for the exposed and unexposed counties may result in differential exposure misclassification in this study; however, neither the magnitude nor the direction of any resulting bias can be estimated.

Age-specific migration rates may be more important determinants of the extent of exposure misclassification than the average migration trends described above. Frost [34] and Frost et al. [34,47] argue that migration will bias the results toward the null and suggest that the relationships between age-specific migration rates, the mode of carcinogenesis and the latency of bladder cancer must all be considered. They argue that if arsenic were an initiator of cancer, then the latency period would be long (20-30 years), and the effect of migration on calculated risks would be large. In contrast, if arsenic is a late-stage promoter of cancer and the latency period is shorter (10-15 years), then the effect of migration on calculated risks will be small.

This follows from the fact that most bladder cancer affects people older than 65 (see Figure 3) and that migration rates decline dramatically with age[34].

Table 10. Population Changes and Bladder Cancer Mortality in Selected New Mexico Counties (1970 – 2000).

County Name	Death Count (1982 - 1998)	Total person-yr (1982-1998)	Population 2000	Population change 1970 - 2000	% change (1970 - 2000)
Exposed Counties					
Bernalillo	143	3,693,973	556,678	240,904	76
Rio Arriba	11	244,797	41,190	16,020	64
Sandoval	21	403,245	89,908	72,416	414
Socorro	8	113,529	18,078	8,315	85
Valencia	17	509,622	66,152	45,684	232
Total	**200**	**4,965,166**	**772,006**	**383,339**	**99**
Total w/out Bernalillo	**57**	**1,271,193**	**215,328**	**142,435**	**195**
Unexposed Counties					
Lincoln	7	111,891	19,411	11,851	156
McKinley	2	145,896	74,798	31,590	73
Mora	1	37,939	5,189	507	11
Otero	13	391,107	62,298	21,201	52
San Juan	22	504,935	113,801	61,284	117
San Miguel	11	216,700	30,126	8,175	37
Sierra	9	81,020	13,270	6,081	85
Taos	6	182,045	29,979	12,463	71
Total	**71**	**1,671,533**	**348,872**	**153,152**	**78**

Data source: Zhan[46]

Confounding and Interaction

The estimated bladder cancer risks should be evaluated in light of other possible confounding factors. These include confounding due to unrecognized differences between the exposed and exposed populations in smoking prevalence, the magnitude of arsenic exposures from other sources, diet, or other socio-economic factors. In addition, it is possible that smoking may an effect modifier, i.e., an interaction between smoking and exposure to arsenic may potentiate bladder cancer.

Smoking Prevalence

A consistently strong relationship between smoking and bladder cancer has been established in a number of studies[10,11]. The relative risk for smokers ranges from 1.0 to more than six depending on duration of smoking, time since smoking cessation, average daily cigarette

consumption, tobacco type and inhalation behavior. The relationship between cigarette smoking and bladder cancer also may be confounded or modified by occupational exposures [5,16].

Smoking prevalence could be a confounder in this study if there is a relationship between smoking prevalence and exposure to arsenic in drinking water at the individual or ecological level. Data describing smoking prevalence in these populations were not available for this study; therefore, other types of data were evaluated for evidence that smoking prevalence differed between arsenic-exposed and unexposed populations. Ethnicity, income, and education were of particular interest because these factors are often associated with smoking prevalence as discussed below.

Differences in the demographic characteristics of the exposed and unexposed population are illustrated in Tables 2-4. As discussed previously, the exposed population in New Mexico had a higher percentage of Hispanics among white males age 20 – 85 years old, than the unexposed populations drawn from adjacent counties (38.1% vs. 33.4%). If Bernalillo County is removed from the exposed population, the contrast is even greater (45.3% vs. 33.4%). Several studies have shown that although smoking rates among Hispanics vary by ethnicity, (e.g., Mexican, Cuban or Puerto Rican), smoking rates among Hispanic men are generally lower than among white men[48,49,50,51]. Those observations are consistent with the results of univariate and logistic regression analyses of the relationship between smoking prevalence and demographic variables in the white male population of New Mexico in the 2000 Behavioral Risk Factor Surveillance System (BRFSS) database described in Attachment B to this report. The analysis showed that the odds of smoking (as defined by the lifetime smoking of 100 cigarettes (5 packs or more) were lower among Hispanic white males than among non-Hispanic white males. The adjusted odds ratio from the logistic regression was 0.6 (95% CI: 0.40 - 0.8) using non-Hispanic white males as the reference group. These trends in smoking prevalence and the difference in ethnicity between exposed and unexposed populations could lead to an ecological association between smoking and exposure to arsenic. This association would result in a negative confounding of the relationship between arsenic exposure and bladder cancer in this study of populations in New Mexico (Tables 6 and 8).

Alternatively, age-specific smoking rates may be more strongly associated with bladder cancer risk. Smoking prevalence among Hispanic males has decreased dramatically since 1978, reflecting the general decrease of smoking in US populations [52]. This means that current or recent smoking prevalence may not reflect rates during the time of arsenic exposure. Nationally, the cigarette smoking rate among older Hispanics is currently lower than among younger Hispanic adults; however, the likelihood of being a former smoker increases with age [49]. Hispanics aged 70 and over are much more likely to be former smokers than those aged 65 - 69 or younger. Attachment B shows that in the 2000 NM BRFSS data set, older (45 - 64 years old) Hispanics whites males smoked more than non-Hispanics white males of the same age, however, the differences were not statistically significant. Differences in age-specific smoking rates between the exposed and unexposed populations during or prior to arsenic exposures cannot be determined from these studies. However, it is possible that during the time of exposure responsible for the bladder cancers observed in 1982- 1998, Hispanic males smoked more than non- Hispanic white males in the study population. This trend and the differences in ethnicity between the exposed and unexposed populations in New Mexico could lead to positive confounding of the relationship between arsenic exposure and bladder cancer.

25

It is also possible that smoking is not a significant confounder in this study. A number of ecological studies suggest that at higher levels of arsenic exposure, excess bladder cancer risks can occur independent of smoking status. Hopenhayn-Rich et al. [26] found statistically significant elevated risks for bladder cancer at drinking water arsenic exposure levels of >40 ppb to 433 ppb in Argentina (Table 1). Deaths from chronic obstructive pulmonary disease were not elevated in these populations, indicating that smoking was not likely to be a confounder. Smith et al. [25] found elevated mortality due to bladder cancer in low-smoking populations in Chile. Chen et al. [20] discounted the possibility that differences in smoking rates (40% vs. 32%) were responsible for higher bladder cancer rates in males (SMR = 11.0; 95% CI = 9.33 – 12.67) in BFD endemic areas of southwest coastal Taiwan compared to the reference population. Chiou *et al*. [53] found elevated risks for bladder cancer in a population in the BFD endemic area of Southwest Taiwan when the results were adjusted for cigarette smoking. However, the results were not statistically significant and the arsenic exposure estimates were not precise.

Effect Modification by Smoking

Smoking trends years after arsenic exposure might be an effect modifier of the relationship between arsenic exposure and bladder cancer, especially if arsenic is an initiator and smoking is a promoter of bladder cancer. Several researchers suggest that risks from low levels of arsenic exposure may be greater among smokers than nonsmokers; however, results from epidemiological studies are not consistent. Bates *et al*. [27] observed an increased risk for bladder cancer only among smokers who have been exposed to arsenic 20-29 years and 30-39 years prior to death. Kurttio et al. [42] found elevated risks for bladder cancer only among smokers in Finland who had been exposed to arsenic concentrations above 0.5 ppb, three to nine years prior to cancer diagnosis (short latency period group). However, in both studies, the high relative risks at such low water-borne arsenic exposures were unexpected and the researchers suggested that confounding and bias might have been significant. Chiou et al. [24] felt that their results "seem to suggest that smoking might play a role in the initiation of arsenic-induced cancer of the urinary organs," but did not explain this suggestion. Most recently, in a case control study of populations exposed to arsenic concentrations of about 100 ppb, Steinmaus et al. [4], found increased risk for bladder cancer only among smokers whose arsenic intake was greater than 80 μg/day, 40 or more years ago.

Attachment A contains a literature review of the biological evidence for synergistic interactions between arsenic and smoking in increasing bladder cancer risk. It is likely that arsenic carcinogenesis results from a combination of processes including chromosomal damage, oxidative stress, and augmentation of growth factors. Several nitrogen compounds in cigarette smoke (N-nitrosoamines and 2-naphthylamine) have been identified as bladder carcinogens[10]. The large number of potential carcinogenetic mechanisms for arsenic and arylamines make it possible that synergistic effects for bladder cancer exist between arsenic and smoking. The literature review failed to identify any studies that definitively demonstrate such synergistic interactions; however, the available information does suggest that arsenic might interfere with detoxification mechanisms for arylamines. Individuals with genetic polymorphisms, which lead to impaired detoxification reactions or DNA repair ability, might be at increased risk to bladder cancer associated with smoking and arsenic exposure[2,54-58]. Mechanisms where synergism might be important include: N-acetylation of arylamines in the liver, O-acetylation of N-hydroxy arylamine metabolites in the bladder lumen, and the activity of glutathione s-transferase. These are described in more detail in Attachment A.

The literature review is not comprehensive with respect to toxic chemical components of cigarette smoke; it was limited primarily to the arylamines. Cigarette smoke contains a large number of chemicals that have not been identified as either initiating agents or promoters but could act as co-carcinogens for arsenic. Conversely, arsenicals could act as co-carcinogens for carcinogens in cigarette smoke. Synergistic interactions may exist that do not involve the mechanisms summarized in this study.

Other Potential Confounders

Although smoking prevalence is likely to be the strongest confounder, other risk factors may be confounders. Other sources of arsenic include occupational exposures, ingestion of arsenic in food and soils, and inhalation of As-rich particulates. Bladder cancer risk has been also related to several dietary habits and differences in diet between the exposed and unexposed populations could introduce confounding. Steimaus et al. [7] found statistically significant associations between increased bladder cancer risk and diets low in vegetable intake (RR = 1.4, 95% CI: 1.08 - 1.83), low in fruit intake (RR = 1.16; 95% CI:1.01 - 1.34), and high in fat intake (RR = 1.37; 95% CI:1.16 - 1.62). Other suggested but unconfirmed risk factors include coffee consumption, alcohol consumption and saccharine use[13]. One study showed that people who drank at least 11 cups of water a day were ½ as likely to develop bladder cancer than a control group who drank less than 6 cups/day [59]. The effects of the above potential confounders and many others were not evaluated directly in this study. Instead, as discussed previously, an attempt to evaluate the potential importance of unrecognized confounders was made by carrying out risk calculations for different subsets of the exposed population and by using alternate reference populations.

Significance for Public Health

There is significant disagreement whether excess bladder cancer risks can be detected in the United States at the relatively low arsenic concentrations in domestic drinking supplies, given uncertainties in exposure levels and potential loss of cases due to migration[2,47]. According to the US EPA[60], the reduction of the arsenic MCL to 10 ppb will prevent approximately 2.3 to 5.5 deaths from bladder cancer and 4.6 to 27.5 deaths from lung cancer each year in the United States. The range in these estimates is due to the uncertainties in the dose-response curve, the ratio of expected bladder cancer to lung cancer deaths, and potential synergistic effects between exposure to arsenic and other risk factors for bladder cancer[2,18,47]. The ecological study described in this paper demonstrates that at low levels of exposure, the calculated risks are also very dependent on the choice of reference population.

This uncertainty in estimated health effects contributes to a wide range in the calculated cost/benefit ratio for the new MCL for arsenic. The projected annual national compliance cost of implementing the new 10 ppb standard ranges from $165 million, estimated by the US EPA [60], to $605 million, estimated by American Water Works Association Research Foundation (AwwaRF) [32]. Frost et al. [61] summarize the incremental costs and benefits of implementing a reduction of the arsenic MCL from 50 ppb to 20 ppb and from 20 ppb to 10 ppb, using the cost estimates from US EPA[60] and AwwaRF[32]. Based on those calculations, the estimates of the cost of the implementing the new 10 ppb arsenic standard range from approximately $5 million to $23.9 million per life saved. Using the incremental costs estimated by the AwwaRF[32], the benefits calculated by Frost et al. [61], and assuming an average 13 years saved per life, the costs

per year of life saved by the 10 ppb MCL are calculated to range from $1,382,717 to $6,612,998. These exceed the value obtained by the US EPA "willingness to pay" method several fold and also are many times higher than the values used by other medical organizations in making determinations about the acceptable costs for certain treatments [60,61,62]

Potential transportation or occupational hazards associated with implementation of the new arsenic standard add additional uncertainty to the cost/benefit ratio. Frost [63] found that if a multi-stage Weibull model was used for the risk estimate and ion exchange was used as the treatment process, then the estimated traffic deaths (1.4 deaths over 70 years) for Albuquerque, New Mexico would nearly equal the 2 cancer deaths averted. The economic impacts of the revised arsenic MCL on rural communities may further reduce the effective health benefit. For example, if the 10 ppb EPA MCL for arsenic in drinking water is enforced by the New Mexico Environment Department, monthly water bills for households in small communities in Sandoval, Bernalillo and Santa Fe Counties could reach $100[64].

The effectiveness of the reduction of the arsenic MCL for reducing bladder cancer rates should be compared to other possible public health interventions. These include reduction of other risk factors for bladder cancer and bladder cancer screening programs. Table 11 compares the bladder cancer risks associated with arsenic exposure to those associated with other risk factors. It can be seen that the highest significant risks (SIR = 1.16 – 1.20) calculated in this study for low level arsenic exposures (10 – 50 ppb) are considerably less than those associated with cigarette smoking; they are similar to those associated with diet and a practice of drinking large volumes of water every day. The additive effect of reducing smoking prevalence and arsenic exposures on bladder cancer rates should be considered. Frost et al. [61] point out that if exposures to low concentrations of arsenic are associated with bladder cancer only among smokers, then the societal risk posed by arsenic in drinking water should be reassessed. Interventions targeting smoking will eliminate both bladder cancer and lung cancer and are likely more cost effective than the reduction in the arsenic MCL, given the treatment costs described above. If detected early, bladder cancer can be cured. The American Cancer Society estimates that the 5-yr survival rate for early bladder cancer is 94%; after it spreads to distal regions, the survival rate is 6%[5]. Screening for bladder cancer in high-risk populations such as smokers or exposed workers is a cost-effective method to reduce bladder cancer mortality rates.

Table 11. Comparison of Bladder Cancer Risks Observed for Different Risk Factors.

Factor	Measure	Value or range	Source
Smoking 20 – 40 cigarettes daily	Incidence odds ratios	3 - 7	Clavel *et al.* [10] Morrison *et al.* [11]
Drinking <6 cups of water daily instead of >11 cups per day	Incidence odds ratio	2	Michaud et al. [59]
Diets low in fruits and vegetables and high in fat	Incidence odds ratio	1.16 – 1.40	Steimaus et al. [7]
Drinking water with arsenic concentration 178 – 780 ppb	Mortality (SMR)	2.16 – 11.0	Several studies listed in Table 1 of this paper
Drinking water with arsenic concentration 10- 50 ppb in NM	Mortality (SMR)	1.08[‡] – 1.16[‡]	Table 6, this study
Drinking water with arsenic concentration 10- 50 ppb in NM	Incidence (SIR)	1.16 – 1.20	Table 8, this study

[‡] not significant at 95% confidence level.

Conclusions

This study demonstrates the effects of the choice of the reference population on the calculated standardized risks (SMRs and SIRs) in ecological studies of bladder cancer and exposure to arsenic in drinking water. When exposed populations drawn from 36 counties nationwide were compared to reference populations based on US standard rates, the SMR was significantly less than 1.0. When compared to reference populations drawn solely from adjacent unexposed counties, the SMR was higher but not statistically significant. A similar pattern was observed when exposed populations in New Mexico and Utah individually were compared to the rates in US standard population, the host state or reference populations drawn from adjacent unexposed counties within the state. None of the calculated SMRs were significantly greater than 1.0, regardless of the reference population that was used. Standardized incidence ratios also showed a dependence on the choice of reference population. For exposed populations in New Mexico, the SIR was significantly less than 1.0 when the rates from the US standard population were used as the reference but was significantly greater than unity when the adjacent unexposed counties were used as a reference population (SIR =1.20; 95% CI =1.13 - 1.26). For Utah, the SIR was also significantly less than 1.0 when the reference population was based on the US standard population. The SIRs were higher when the reference population was based on state rates or the adjacent unexposed counties but were not statistically significant.

The results demonstrate the uncertainties in estimates of the bladder cancer risks to US populations due to arsenic in drinking water obtained from ecological studies. Differences between the exposed and unexposed populations, such as smoking prevalence prior to and during arsenic exposure, and factors related to differences in urbanization could be sources of confounding. Given the estimated high costs associated with implementation of the new MCL for arsenic, additional research to reduce these uncertainties is warranted. Studies of smoking histories of the exposed and unexposed populations and the use of age-specific migration rates to place bounds on misclassification due to migration might lead to more reliable estimates of arsenic-related bladder cancer risk. Additional research should be carried out to establish the shape of the dose-response curve at low concentrations (10 – 50 ppb) and to identify synergistic effects of arsenic exposure and other carcinogens. As an alternative or in addition to the reduction of the arsenic MCL, other possible interventions for bladder cancer should be considered. These include 1) increased screening of high-risk populations for early stage bladder cancer and 2) reducing smoking prevalence by anti-smoking legislation and campaigns.

References

1. U.S. Environmental Protection Agency. National primary drinking water regulations; Arsenic and clarifications to compliance and new source contaminants monitoring. Federal Register 2000; 65(121):38887-38983.

2. National Research Council. Arsenic in Drinking Water: 2001 Update. Washington, DC 20055: National Academy Press, 2001.

3. Lewis DR, Southwick JW, Ouellet-Hellstrom R, Rench J, Calderon RL. Drinking water arsenic in Utah: A cohort mortality study. Environmental Health Perspectives 1999;107(5):359-366.

4. Steinmaus CM, Yuan Y, Bates MN, Smith AH. Case-control study of bladder cancer and drinking water arsenic in the western United States. American Journal Epidemiology 2003;158:1193-1201.

5. Bladder Cancer Resource Center. Retrieved July 21, 2003, from American Cancer Society: http://www.cancer.org/docroot/CRI/content/CRI_2_4_1X_What_are_the_key_statistics_for_bladder_cancer_44.asp?sitearea=&level=.

6. Bladder Cancer. Retrieved July 21, 2003, from American Foundation for Urologic Disease: http://www.afud.org/conditions/bc.html.

7. Steinmaus CM, Nunez S, Smith AH. Diet and bladder cancer: a meta-analysis of six dietary variables. American Journal Epidemiology 2000;151(7):693-702.

8. Miller BA, Kolonel LN, Bernstein L, Young JJL, Swanson GM, West D, Key CR, Liff JM, Glover CS, Alexander GA, eds. Racial/Ethnic Patterns of Cancer in the United States 1988-1992 NIH Pub. No. 96-4104. Bethesda, MD: National Cancer Institute, 1996.

9. Ries LAG, Eisner MP, Kosary CL, Hankey BF, Miller BA, Clegg L, Edwards BK. SEER Cancer Statistics Review 1973 - 1997. Bethesda, MD: National Cancer Institute, 2000.

10. Clavel J, Cordier S, Boccon-Gibod L, Hemon D. Tobacco and bladder cancer in males: increased risk for inhalers and smokers of black tobacco. International Journal Cancer 1989;44:605-610.

11. Morrison AS, Buring JE, Verhoek WG, Aoki K, Leck I, Ohno Y, Obata K. An international study of smoking and bladder cancer. The Journal of Urology 1984;131:650-654.

12. Gerhke S, Kalbe T, Staehler G. Bladder cancers a model tumor of chemically induced carcinogenesis: A historical review. Aktuelle Urologie 1996;27(4): 218-220.

13. Urinary Bladder - US Racial/Ethnic Patterns. Retrieved July 21, 2003, from National Cancer Institute: http://www.cancer.gov/statistics/cancertype/urinary-racial-ethnic.

14. Smith AH, Hopenhayn-Rich C, Bates MN, Goeden HM, Hertz-Picciotto I, Duggan HM, Wood. R., Kosnett MJ, Smith MT. Cancer risks from arsenic in drinking water. Environmental Health Perspectives 1992;97:259-267.

15. Bates MN, Smith AH, Hopenhayn-Rich C. Arsenic ingestion and internal cancers: a review. American Journal of Epidemiology 1992; 135(5):462-476.

16. Siemiatycki J, Dewar R, Nadon L, Gerin N. Occupational risk-factors for bladder cancer: Results from a case-control study in Montreal, Quebec, Canada. American Journal Epidemiology 1994;140(12):1061-1080.

17. Kitchin KT. Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites. Toxicology and Applied Pharmacology 2001;172:249-261.

18. National Research Council. Arsenic in Drinking Water. Washington, DC 20055: National Academy Press, 1999.

19. Welch A, Lico MS, Hughes JL. Arsenic in groundwater of the western United States. Groundwater 1988;26:333 - 347.

20. Chen CJ, Chuang YC, Lin TM, Wu HY. Malignant neoplasms among residents of a blackfoot disease-endemic area in Taiwan: High-arsenic artesian well water and cancers. Cancer Research 1985;45:5895-5899.

21. Chen CJ, Wang CJ. Ecological correlation between arsenic level in well water and age-adjusted mortality from malignant neoplasms. Cancer Research 1990;50:5470-5474.

22. Tsai S-M, Wang TN, Ko Y-C. Mortality for certain diseases in areas with high levels of arsenic in drinking water. Archives of Environmental Health 1999;54(3):186-193.

23. Guo HR, Chang H, Lipsitz SR, Monson RR. Arsenic in drinking water and incidence of urinary cancers. Epidemiology 1997;8:545-550.

24. Chiou HY, Chiou ST, Hsu YH, Chou YL, Tseng CH, et al. Incidence of Transitional Cell Carcinoma and Arsenic in Drinking Water: A Follow-Up Study of 8,102 Residents in an Arseniasis-Endemic Area in Northeastern Taiwan. American Journal of Epidemiology 2001;153:411- 418.

25. Smith AH, Goycolea M, Haque R, Biggs ML. Marked increase in bladder and lung Cancer mortality in a region of northern Chile due to arsenic in drinking water. American Journal Epidemiology 1998;147:660-669.

26. Hopenhayn-Rich C, Biggs ML, Fuchs A, Bergoglio R, Tello EE, Nicolli H, Smith AH. Bladder cancer mortality associated with arsenic in drinking water in Argentina. Epidemiology 1996;7:117-124.

27. Bates MN, Smith AH, Cantor KP. Case-control study of bladder cancer and arsenic in drinking water. American Journal of Epidemiology 1995;141:523-530.

28. Frost FJ. Arsenic Data Summary Report Submitted to:American Water Works Association Research Foundation. Albuquerque, NM: Lovelace Respiratory Research Institute, 2001.

29. U.S. Environmental Protection Agency. Arsenic Occurrence in Public Drinking Water Supplies. Washington, D.C.: Office of Ground Water and Drinking Water, Environmental Protection Agency, 2000.

30. Focazio MJ, Smith AH, Watkins SA, Helsel DR, Horn MA. A Retrospective Analysis on the Occurrence of Arsenic in Ground-water Resources of the United States and Limitations in Drinking-Water Supply Characterizations, Water Resources Investigations Report 99-4279. Reston, VA: US Geological Survey, 2000.

31. Arsenic and Old Laws: An Analysis of Arsenic Occurrence in Drinking Water. Retrieved July 21, 2003, from Natural Resources Defense Council: http://www.nrdc.org/water/drinking/arsenic/aolinx.asp.

32. Frey MM, Chwirka J, Narasimhan R, Kommineni S, Chowdhury Z. Cost Implications of a Lower Arsenic MCL Final Report. American Water Works Research Foundation, 2000.

33. Engel RR, Smith AH. Arsenic in drinking water from mortality from vascular disease: an ecologic analysis in 30 counties in the United States. Archives of Environmental Health 1994;49:418 - 427.

34. Frost FJ. Arsenic Data Summary Report Submitted to: American Water Works Association Research Foundation. Albuquerque, NM: Lovelace Respiratory Research Institute, 2002.

35. Frost F, Muller T, Petersen H, Thomson B, Tollestrup K. Identifying US populations for the study of health effects related to drinking arsenic. Journal of Exposure Analysis and Environmental Epidemiology 2003;13:231-239.

36. SEERSTAT. Retrieved July 21, 2003, from National Cancer Institute: http://www.seer.cancer.gov.

37. USA Counties 1998. Retrieved January 5, 2002, from Oregon State University - Information Services: http://govinfo.kerr.orst.edu/usaco-stateis.html.

38. U.S. Environmental Protection Agency. Arsenic Occurrence in Public Drinking Water Supplies. Washington, D.C.: Office of Ground Water and Drinking Water, Environmental Protection Agency, 2000b.

39. State and County Quick Facts. Retrieved March 12, 2002, from U.S. Census Bureau: http://www.census.gov/main/www/access.html.

40. WONDER Database. Retrieved July 21, 2003, from Center for Disease Control: http://wonder.cdc.gov.

41. International Statistical Classification of Diseases, Injuries, and Causes of Disease, Ninth Revision. Geneva: World Health Organization, 1977.

42. Kurttio P, Pukkala E, Kahelin H, Auvinen A, Pekkanen J. Arsenic concentrations in well water and risk of bladder and kidney cancer in Finland. Environmental Health Perspectives 1999;107(9):705 - 710.

43. Morgenstern H. Ecologic studies in epidemiology: Concepts, principles, and methods. Annual Review of Public Health 1995;16:61-81.

44.   Piantadosi S, Byar DP, Green SB. The ecological fallacy. American Journal of Epidemiology 1988;127:893 - 904.

45.   Screening for bladder cancer. In: DiGuiseppi C, Atkins D, Woolf DB, eds. U.S. Preventive Services Task Force: Guide to Clinical Preventive Services, 2nd ed. Alexandria, VA: International Medical Publishing, 1996;181-186.

46.   Zhan FB. Childhood cancer clusters in New Mexico, 1973-1997. Southwestern Geographer 2001;5:31-48.

47.   Frost F, Craun G, Brown KG. Detection of excess arsenic-related cancer risks. Environmental Health Perspectives 2002;110(1):A12-A13.

48.   US Department of Health and Human Services. Tobacco use among US racial/ethnic minority groups - African Americans, American Indians and Alaska Natives, Asian Americans and Pacific Islanders, and Hispanics: a report of the Surgeon General. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 1998.

49.   Minority Smoking Factsheet. Retrieved June 1, 2003, from Center for Social Gerontology: http://www.tcsg.org/tobacco/minorities/hamsheet.htm.

50.   Minority Smoking Factsheet. Retrieved June 1, 2003, from American Lung Association: http://www.lungusa.org/pub/minority/lungcancer_00.html.

51.   Tobacco information and prevention source (Tips) - Hispanics and tobacco. Retrieved June 1, 2003, from National Center for Chronic Disease Prevention and Health Promotion: http://www.cdc.gov/tobacco/sgr/sgr_1998/sgr-min-fs-hsp.htm.

52.   American Cancer Society. Cancer Facts and Figures for Hispanics, 2000-2001. Atlanta, GA: American Cancer Society, 2001.

53.   Chiou HY, Hsueh YM, Liaw KF, Horng SF, M.H. Chiang, Pu YS, Lin JS, Huang CH, Chen. CJ. Incidence of internal cancers and ingested inorganic arsenic: A seven-year follow-up study in Taiwan. Cancer Research 1995;55:1296-1300.

54.   Badawi AF, Hirvonen A, Bell DA, Lang NP, Kadlubar FF. Role of aromatic amine acetyltransferases, NAT1 and NAT2, in carcinogen-DNA adduct formation in human urinary bladder. Cancer Research 1995;55:5230-5237.

55.   Chiou HY, Hsueh YM, Hsieh LL, Hsu LI, Hsu YH, et al. Arsenic methylation capacity, body retention and null genotypes of glutathione S-transferase M1 and T1 among current arsenic-exposed residents in Taiwan. Mutation Research 1997;386:197-207.

56.   Rebbeck TR. Molecular epidemiology of the human glutathione S- transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiology, Biomarkers & Prevention 1997(6):733-743.

57.   Su JH, Gou YL LM, Huang JD, Cheng Y, Christiani DC. The NAT2* slow acetylator genotype is associated with bladder cancer in Taiwanese but not in the Black Foot Disease endemic area population. Pharmacogenetics 1998;8:187-190.

58.   Taylor JA, Umbach DM, Stephens E, Castranio T, Paulson D, Robertson C, Mohler JL, Bell BA. The role of N-acetylation polymorphisms in smoking-associated bladder cancer:

evidence of a gene-gene-exposure three-way interaction. Cancer Research 1998;58:3603-3610.

59.   Michaud DS, Giovannucci EL, Spiegelman D, Clinton SK, Rimm EB, Willett WC. Total fluid intake and the risk of bladder cancer in men. American Journal of Epidemiology 1999;147(11):173-173.

60.   U.S. Environmental Protection Agency. National primary drinking water regulations; Arsenic and clarifications to compliance and new source contaminants monitoring. Federal Register 2000a;65(121):38887-38983.

61.   Frost F, Tollestrup K, Craun G, Raucher R, Stomp J, Chwirka J. Evaluation of costs and benefits of a lower arsenic MCL. Journal of the American Water Works Association 2002;94(3):71-80.

62.   Appendix I: Valuation of human health and welfare effects of criteria pollutants. Retrieved July 1, 2000, from U.S. Environmental Protection Agency: www.epagov/oar/sect812/copy.html.

63.   Frost F. Arsenic- New Mexico's challenge -. New Mexico Environmental Health Conference Short Course Notes 2001.

64.   Bitner KA. Cost of Compliance with a Lower Drinking Water Standard in New Mexico [Masters]. University of New Mexico, 2001.

Appendix I. Bladder Cancer Mortality Rates and Arsenic Levels in Exposed Counties

State	County Name	Death Count	Total person-yr (1982-1998)	*Age Adjusted Rate	*Crude Death Rate	As Level (ppb)
AZ	PINAL	63	888,740	7.5	7.09	10.3
CA	KINGS	18	768,083	4.6	2.34	16.1
CA	MONO	3	84,969	9.3	3.53	13
CO	ALAMOSA	8	110,095	10.4	7.27	36.9
CO	RIO GRANDE	5	91,921	5.5	5.44	23.8
ID	PAYETTE	10	143,111	7.1	6.99	14.4
ID	WASHINGTON	6	72,888	6.8	8.23	17
ID	TWIN FALLS	38	303,656	8.7	12.51	*22.51*
IL	DE WITT	10	140,170	8.1	7.13	17.1
ND	DIVIDE	2	24,555	4.2	8.14	13.6
ND	LA MOURE	5	46,932	9.2	10.65	14.9
NM	BERNALILLO	143	3,693,973	6.6	3.87	14.1
NM	RIO ARRIBA	11	244,797	5.9	4.49	17
NM	SANDOVAL	21	403,245	9	5.21	17
NM	SOCORRO	8	113,529	10.6	7.05	32.2
NM	VALENCIA	17	509,622	5	3.34	16
NV	CHURCHILL	11	142,550	8.6	7.72	90
NV	ELKO	5	266,586	4.7	1.88	16
NV	ESMERALDA	1	11,369	7.8	8.8	25.6
NV	LANDER	2	49,134	10.6	4.07	17.3
NV	LINCOLN	0	31,014	0	0	15.7
NV	LYON	5	173,514	4.1	2.88	21.3
NV	NYE	11	160,750	9.5	6.84	13.6
OK	CANADIAN	26	588,742	7.9	4.42	26.9
OK	CIEVELAND	40	1,351,709	7	2.96	35
OK	CUSTER	9	206,543	5	4.36	13
TX	ANDREWS	5	119,854	6.8	4.17	33.6
TX	BORDEN	0	6,893	0	0	22
TX	GAINES	4	118,424	6.1	3.38	12.1
TX	HUDSPETH	0	25,379	0	0	11.6
TX	JIM HOGG	1	42,886	3.2	2.33	77.9
TX	KARNES	4	101,350	3.7	3.95	15.6
TX	MIDLAND	20	827,105	4.4	2.42	20
TX	YOAKUM	1	73,938	2.5	1.35	11.7
UT	SUMMIT	2	149,654	4.3	1.34	12.6
UT	MILLARD	7	99,182	9	7.06	14-166

*rates per 100,000 person years. Populations, deaths, crude rates and age-adjusted rates from CDC[1].
Age- adjusted rates are calculated using the 2000 US Standard population.

1. Center for Disease Control. WONDER Database. 2001.

Appendix II. Unexposed Counties for This Study (As < 10 ppb in drinking water)

STATE	FIPS Code	County Name	Death Count	Population	*Age Adjusted Rate	*Crude Death Rate
AZ	4003	COCHISE	48	784,623	8.2	6.12
AZ	4007	GILA	23	299,603	6	7.68
AZ	4009	GRAHAM	8	196,161	5.1	4.08
AZ	4015	MOHAVE	93	782,672	9.8	11.88
AZ	4027	YUMA	55	957,944	5.8	5.74
CA	6003	ALPINE	1	8,235	10.3	12.14
CA	6027	INYO	13	135,531	7.9	9.59
CA	6039	MADERA	46	737,707	8.4	6.24
CA	6043	MARIPOSA	10	113,807	6.6	8.79
CA	6079	SAN LUIS OBISPO	97	1,669,661	7	5.81
CA	6109	TUOLUMNE	33	382,352	9	8.63
CO	8007	ARCHULETA	3	49,598	10.1	6.05
CO	8021	CONEJOS	0	64,070	0	0
CO	8023	COSTILLA	0	27,200	0	0
CO	8055	HUERFANO	3	52,334	3.7	5.73
CO	8067	LA PLATA	14	274,911	10.4	5.09
CO	8109	SAGUACHE	1	40,261	4	2.48
ID	16003	ADAMS	5	30,181	14.7	16.57
ID	16031	CASSIA	10	170,970	7.6	5.85
ID	16039	ELMORE	9	185,510	10.5	4.85
ID	16045	GEM	3	104,615	2.2	2.87
ID	16047	GOODING	10	105,023	8.7	9.52
ID	16053	JEROME	10	135,391	10.8	7.39
ID	16063	LINCOLN	5	30,062	16.3	16.63
ID	16067	MINIDOKA	14	167,362	11.2	8.37
ID	16073	OWYHEE	2	74,774	2.6	2.67
ID	16085	VALLEY	6	58,687	12.3	10.22
IL	17053	FORD	12	116,654	9.1	10.29
IL	17107	LOGAN	18	245,854	7.5	7.32
IL	17147	PIATT	6	132,720	5.5	4.52
ND	38003	BARNES	8	104,367	6.1	7.67
ND	38013	BURKE	5	26,012	14.1	19.22

* rates per 100,000 person years. Populations, deaths, crude rates and age-adjusted rates from CDC[1].
Age- adjusted rates are calculated using the 2000 US Standard population.

1.   Center for Disease Control. WONDER Database. 2001.

Appendix II. Unexposed Counties for This Study (As < 10 ppb in drinking water)
(continued)

STATE	FIPS Code	County Name	Death Count	Population	*Age Adjusted Rate	*Crude Death Rate
ND	38047	LOGAN	4	24,579	10.2	16.27
ND	38051	MC INTOSH	7	33,187	10.5	21.09
ND	38061	MOUNTRAIL	4	50,004	5.5	8
ND	38073	RANSOM	5	52,704	6.8	9.49
ND	38081	SARGENT	4	41,113	10.6	9.73
NM	35027	LINCOLN	7	111,891	7.8	6.26
NM	35031	MC KINLEY	2	145,896	1.5	1.37
NM	35033	MORA	1	37,939	2.5	2.64
NM	35035	OTERO	13	391,107	4.2	3.32
NM	35045	SAN JUAN	22	504,935	6.9	4.36
NM	35047	SAN MIGUEL	11	216,700	6.8	5.08
NM	35051	SIERRA	9	81,020	5.3	11.11
NM	35055	TAOS	6	182,045	4.3	3.3
NV	32510	CARSON CITY	22	331,733	9	6.63
NV	32005	DOUGLAS	14	233,363	7.1	6
NV	32011	EUREKA	1	13,198	13.5	7.58
NV	32013	HUMBOLDT	3	112,749	4.6	2.66
NV	32021	MINERAL	2	43,101	3.1	4.64
NV	32027	PERSHING	1	35,526	2.9	2.81
NV	32029	STOREY	0	19,890	0	0
NV	32033	WHITE PINE	6	77,736	9.4	7.72
OK	40009	BECKHAM	11	160,482	6.4	6.85
OK	40015	CADDO	21	193,264	9.7	10.87
OK	40051	GRADY	17	327,885	6.1	5.18
OK	40073	KINGFISHER	12	111,568	11.3	10.76
OK	40081	LINCOLN	10	227,749	4.8	4.39
OK	40083	LOGAN	9	204,560	5.3	4.4
OK	40087	MC CIAIN	8	187,920	6.4	4.26
OK	40125	POTTAWATOMIE	22	426,532	6.2	5.16
OK	40149	WASHITA	10	103,186	9	9.69
OR	41001	BAKER	22	132,524	14.1	16.6
OR	41045	MALHEUR	19	217,153	9	8.75
TX	48047	BROOKS	0	69,517	0	0
TX	48079	COCHRAN	1	34,452	3	2.9
TX	48103	CRANE	3	39,165	13.6	7.66

Appendix II. Unexposed Counties for This Study (As < 10 ppb in drinking water)
(continued)

STATE	FIPS Code	County Name	Death Count	Population	Age Adjusted Rate	Crude Death Rate
TX	48109	CULBERSON	1	28,637	3.7	3.49
TX	48123	DE WITT	12	137,110	6.9	8.75
TX	48135	ECTOR	37	990,876	7.1	3.73
TX	48169	GARZA	2	39,703	5.5	5.04
TX	48173	GLASSCOCK	1	12,374	17.6	8.08
TX	48175	GOLIAD	5	46,341	11.9	10.79
TX	48177	GONZALES	5	132,459	3	3.77
TX	48219	HOCKLEY	8	195,717	9.3	4.09
TX	48227	HOWARD	20	270,565	7.6	7.39
TX	48243	JEFF DAVIS	1	16,785	5	5.96
TX	48297	LIVE OAK	0	80,735	0	0
TX	48305	LYNN	5	56,401	8.5	8.87
TX	48317	MARTIN	0	41,754	0	0
TX	48335	MITCHELL	6	68,972	7.1	8.7
TX	48377	PRESIDIO	1	54,741	1.9	1.83
TX	48383	REAGAN	2	38,304	18.5	5.22
TX	48415	SCURRY	9	153,889	7.3	5.85
TX	48461	UPTON	2	37,684	10.9	5.31
TX	48493	WILSON	6	193,975	5.3	3.09
TX	48495	WINKLER	4	74,837	6.2	5.34
TX	48505	ZAPATA	1	79,168	1.7	1.26
UT	49001	BEAVER	2	41,836	5.2	4.78
UT	49003	BOX ELDER	19	308,939	9.3	6.15
UT	49013	DUCHESNE	3	113,864	4.1	2.63
UT	49021	IRON	9	181,976	9	4.95
UT	49023	JUAB	1	52,061	2	1.92
UT	49029	MORGAN	1	49,320	3.7	2.03
UT	49039	SANPETE	3	145,648	3.1	2.06
UT	49041	SEVIER	2	134,301	1.6	1.49
UT	49045	TOOELE	4	235,307	2.5	1.7
UT	49047	UINTAH	9	183,689	9.7	4.9
UT	49051	WASATCH	1	89,067	1.8	1.12
UT	49053	WASHINGTON	26	430,402	5.7	6.04
total			1088	18,158,662		5.99

Appendix III: Calculation of Standardized Mortality and Incidence Ratios
(SMRs and SIRs)

Mortality data for bladder cancer for the period 1982 to 1998 for white males were obtained from the CDC WONDER Database[1] for IDC9 codes 188 – 188.9. Standardized mortality ratios (SMR) for white males were calculated for the 34 counties with arsenic concentrations higher than 10 pbb (Appendix I, i.e., " the national exposed population") as well as separately for exposed counties in Utah and New Mexico. Reference populations for SMRs were based on US national rates, aggregated rates for the 105 unexposed counties listed in Appendix II. Separate analyses were done for Utah and New Mexico using national rates, state rates, and rates for local unexposed counties in each state. Incidence data for the corresponding SEER topographies were obtained from the SEERSTAT program of the National Cancer Institute[2]. Standardized incidence ratios (SIRs) for exposed population in New Mexico and Utah were calculated using reference populations based on US national rates, state rates for Utah and New Mexico, and the sets of unexposed counties for each state individually.

The following procedure was used:

1.   Total observed numbers of bladder cancer deaths (d_i) and incident cases (c_i) for 10-year age intervals were obtained from the CDC WONDER and SEER databases, respectively, for the time interval 1982 – 1998 for the counties with high arsenic concentrations (>10 ppb). The age-specific numbers of cases and deaths for the entire exposed population were calculated by summing the cases and deaths over all the exposed counties for each age interval.

2.   The age distribution of the exposed population was calculated by summing the values of person-time (person-yr) over all the exposed counties for each age interval.

3.   Reference mortality and incidence rates for the time interval 1982 – 1998 were obtained from the total observed numbers of bladder cancer deaths and incident cases in the unexposed counties for the same decade age intervals. Age-specific incidence and mortality rates for the unexposed (reference) populations were calculated using the corresponding total population x time values in the unexposed counties as the denominators. Age-specific mortality rates were also compiled for host states and the United States for the same time intervals.

4.   Expected numbers of death (D_i) and cases (C_i) for the exposed counties during the time period 1982-1998 were calculated from the age-specific rates in the reference population obtained in (3) and the age distributions for the exposed populations obtained in (2).

5.   The SMR was calculated as:

   $SMR = 100 \times \Sigma(d_i/D_i)$

where D_i is the expected number of deaths from bladder cancers in the exposed population for the ith age-stratum obtained in (4) and d_i is the observed number of deaths in the exposed counties for the corresponding age stratum obtained in (1). The Standardized Incidence Ratio was calculated in a similar manner using expected (C_i) and observed (c_i) numbers of incident cases.

6.   Confidence intervals for the SMRs and SIRs were calculated using standard methods described by Kelsey et al. [3] (p. 177). If the lower limit of the confidence interval for the SMR or SIR is greater than unity, then the risk is considered significantly elevated.

References

1.   Center for Disease Control. WONDER Database. 2001.

2.   National Cancer Institute. SEERSTAT. 2001.

3.   Kelsey JL, Whittemore AS, Evans AS, Thompson WD. Methods in Observational Epidemiology. 2 ed. New York: Oxford University Press, 1996.

Attachment A

Relationships among Smoking, Arsenic Exposure
and Bladder Cancer

Table of Contents

Figures and Tables

Introduction

Bladder cancer is considered to be the model of chemical carcinogenesis [1]. Toxicological studies in the early to mid 20th century established the role of aromatic amines (arylamines) in causing bladder cancer. The use of these compounds in the paint, pesticide, pharmaceutical and chemical industries is associated with increased incidence of bladder cancer. The presence of these compounds in cigarette smoke also may be responsible for the strong association between bladder cancer and smoking. It has been suggested that exposure to metal compounds containing titanium, cadmium, and arsenic may be associated with bladder cancer[2-4] although the mechanism of cancer induction is not known.

In its evaluation of the technical basis for of the proposed EPA Safe Drinking Water Standard for arsenic, the National Research Council[5] concluded that there was no evidence that smoking was a significant confounder of the relationship between arsenic consumption in drinking water and the risk of bladder cancer. The NRC, however, recommended that future studies of the health effects of arsenic exposure consider interactions with host factors that might influence susceptibility to bladder cancer. The lack of consistency among the epidemiological studies concerning possible interactions between smoking and arsenic exposure and the absence of a model for arsenic carcinogenesis are major reasons for this recommendation.

The potential roles of smoking as a confounder and effect modifier of the relationship between arsenic exposure and bladder cancer are important to the ecological study described in this professional paper. The purposes of this attachment are to 1) summarize the independent roles of arsenic exposure and smoking in the development of bladder cancer and 2) describe the evidence that an interaction might exist between arsenic consumption and smoking in development of bladder cancer. Epidemiological studies that demonstrate relationships between arsenic exposure, smoking and bladder cancer are discussed in the main body of the professional paper. An overview of the natural history of bladder cancer is found in this attachment. Next, the probable biological mechanisms by which arsenic and arylamines, individually, cause bladder cancer are described. Finally, those mechanisms through which arsenic and arylamines might synergistically interact to increase the incidence of bladder cancer are suggested.

Natural History of Bladder Cancer

Bladder cancer typically starts in the epithelial tissues of the bladder lining and may involve papillary transitional cells or sessile cells. It spreads by sequentially invading the basement membrane, the muscle layer of the bladder wall and then fatty tissues surrounding the bladder. From there it can invade surrounding organs in the pelvic region such as the prostate, spread through the lymph system and invade more distant organs such as the liver.

The most common staging system to describe the progression of bladder cancer is the TNM system developed by the American Joint Committee on Cancer (AJCC). In this three code system, the letter *T*, followed by a number from 1 to 4 describes the extent of invasion of the tumor into the bladder wall and adjacent tissues; the letter *N* followed by a number from 0- 3,

43

describes spread to nearby lymph nodes; and the letter *M*, followed by a 0 or 1, indicates if the tumor has spread to distant organs. Descriptions of the characteristics of the stages are shown in Table A-1. Topography and morphology of bladder cancer are described by ICD-O code C67; sites are subdivided into 10 categories. These correspond to Revision 9 of the International Classification for Diseases (ICD-9) codes 188 – 188.9 (ICD-9 code N-188 and its 10 subcategories).

Table A-1. Characteristics of Bladder Cancer Stages

Stage	Description
TNM Stage 0a (in situ)	Noninvasive papillary transitional cell carcinoma that has grown towards the center of the bladder but not invaded the basement membrane, muscle or connective tissue of the bladder wall.
TNM Stage 0is (in situ)	Flat (sessile), non-invasive carcinoma involving the mucosa of the bladder but not basement membrane.
TNM Stage I (Localized)	Invasion of layer of connective tissue or supporting tissue but not the thick layer of muscle in the bladder wall.
TNM Stage II (Localized)	Invasion of the muscle layer but has not penetrated the layer to reach surrounding fatty tissue
TNM Stage III (Regional)	Penetration of the thick muscle layer to reach the layer of fatty tissue that surrounds the bladder and may have spread to the prostate, uterus or vagina
TNM Stage IV (Distant)	Cancer has spread through the bladder wall to pelvic or abdominal wall, lymph nodes or distant organs.

Specific Mechanisms of Bladder Carcinogenesis

Overview

A multistage model for bladder cancer has been proposed by a number of workers. In these models, carcinogenic chemicals are classified as initiators and promoters. *Initiation* is the first step in development of a cancer during which the cell's genetic material is damaged in a manner that could lead to subsequent neoplasm development. The initiating event may be the binding of an electrophile to cellular DNA causing a permanent, heritable but unexpressed change in the genome. *Promoting* agents are chemicals that are not carcinogens by themselves but instead act to stimulate cell division and produce a tumor through clonal proliferation. A long time period may elapse between initiation and tumor development. Low chronic doses of the promoting agent will increase cancer incidence by increasing the number of tumors or

decrease the latency period. Chemicals that act as both initiators and promoters are called *complete* carcinogens.

Arsenic has been shown to both a promoter and a complete carcinogen in a number of *in vitro* and *in vivo* studies. In a review of recent research, Kitchin [6] describes the potential roles of nine different possible modes of arsenic carcinogenesis. These include induced chromosomal abnormalities, oxidative stress, altered DNA repair, altered DNA methylation patterns, altered growth factors, enhanced cell proliferation, promotion/progression, gene amplification, and suppression of p53. He suggests that three modes of arsenic carcinogenesis have the most evidence: chromosomal abnormalities, oxidative stress and altered growth factors.

Several nitrogen compounds in cigarette smoke (N-nitrosoamines and 2-naphthylamine) have been identified as bladder carcinogens[7]. There is epidemiological and experimental evidence that aromatic amines in cigarette smoke are causally related to DNA adduct formation in bladder epithelial cells. Formation of these adducts may be initiating events in transitional cell carcinomas which account for about 95% of all bladder cancers[8]. In addition, cigarette smoke contains reactive oxygen species (ROS) such as oxygen radicals, hydroxy radicals, and derivatives of O_2 that lack unpaired electrons (H_2O_2). These chemicals can initiate lipid peroxidations, oxidize proteins and cause damage to DNA directly and indirectly[9].

In the following section, the most important processes by which arsenic and arylamines in cigarette smoke could cause bladder cancer are described. Relatively simple processes involving the interactions between arsenic and proteins are summarized first. The ability of arsenic to form strong chemical bonds with the sulfydryl groups in cysteine residues leads to a large number of possible interactions between As and cellular material. Next, potential pathways through which arsenic and arylamines could inhibit detoxification reactions are described. Many of these interactions result in DNA damage. Recent molecular epidemiological studies that demonstrate gene-environment interactions linking bladder cancer to exposure to arsenic or arylamines are described last. In a subsequent section, several speculations about interactions between arsenic and arylamines and their role on bladder cancer are discussed.

Reactions of Inorganic Arsenic Species

Humans ingest a variety of arsenic species with various oxidation states and complexes. Most arsenic is present in either the trivalent oxidation state or the pentavalent oxidation state. In general, As(III) species are more toxic than As(V) species. The difference in the reactivities of pentavalent AsO_4^{2-} and trivalent AsO_3^{2-} species can be understood by referring to their electronic configuration (As(V) = $[Ar]3d^{10}$; As(III) = $[Ar]3d^{10}4s^2$). Trivalent arsenic species have an unshared 4s electron pair which can bind to biological molecules. Pentavalent arsenic has little direct effect on enzyme activity because it lacks this electron pair.

As(III) forms strong bonds with sulfur, therefore, arsenic binding to the sulfhydryl groups of cysteine is important for a number of protein and enzyme systems. It is estimated that As(III) can bind to and inhibit the activity of at least 200 proteins[5]. The binding inhibits the activities of enzymes such as pyruvate dehydrogenase and 2-oxogluturate dehydrogenase and potentially affects the enzymes produced by the cytochrome P-450 superfamily of genes. The high clastogenicity that lead to chromosomal abnormalities is related to the tendency of trivalent arsenic to disrupt the formation of tubulin and spindles during mitosis.

Arsenic accumulates in the mitochondria and its cellular toxicity and carcinogenicity might be related to its damaging effect on mitochondrial enzymes and tissue respiration[6]. The activity of both As(III) and As(V) are important. Arsenite binds to the dihydrolipoic acid cofactor that is necessary for oxidation of NAD-linked substrates which mediate respiration. In addition, arsenite inhibits succinic dehydrogenase activity and uncouples oxidative phosphorylation, resulting in stimulation of ATPase activity. Arsenic (V) competes with phosphorous during oxidative phosphorylation; As(V)-OR esters are formed by enzymes which build ATP. The product hydrolyzes very rapidly, thereby decoupling oxidative phosphorylation. Arsenic also inhibits energy-linked reduction of NAD. The inhibition of mitochondrial respiration ultimately leads to oxidative stress and production of reactive oxygen species (ROS), which may damage DNA and initiate cancer.

Methylated Arsenicals

The majority of arsenic that is eliminated in urine by humans is in the form of methylated arsenicals. Typically, 10-30% of the arsenic is in inorganic forms, 10-20% is in the form of monomethylarsonic acid (MMA) and 55-75% is excreted as dimethylarsinic acid (DMA)[10]. In the methylated forms of arsenic, MMA and DMA, the electronegative hydroxyl groups are replaced by methyl groups that make the complex more hydrophobic and less ionized. Both As(III) and As(V) species are found in humans; the DMA(V) species is predominant. Methylation of arsenic occurs primarily but not exclusively in the liver and produces a number of As(III) and As(V) intermediates (see Figure A-1). The process includes a number of oxidation, reduction and methylation steps. These involve the activities of S-adenosyl methionine (SAM) and Glutathione (L-γ-glutamyl-L-cysteinylglycine or GSH). GSH acts as reducing agent for As(V) species that accept methyl groups from SAM and is discussed in more detail below. The exact proportion of end products depends on a large number of exposure variables and the site of metabolism, and it varies among different human populations.

The role of arsenic methylation in carcinogenesis is currently unclear. Until recently, it was believed that the methylated forms were relatively nontoxic and that methylation of arsenic was primarily a detoxification mechanism. In addition, it had been suggested that As methylation caused cancer indirectly, by interfering with DNA methylation pathways[11]. In this model, the altered DNA methylation state would lead to altered gene expression and ultimately to carcinogenesis.

Recent studies, however, indicate that the methylated forms of arsenic are both toxic and directly carcinogenic[5,6]. Both DMA(III) and MMA(III) can cause enzyme inhibition, cell toxicity and genotoxicity. Kitchin[6] reviews studies that demonstrate that DMA acts as either as a promoter or complete carcinogen for bladder cancer. In some of these studies, rats initiated with various nitrosoamines were subsequently given arsenic in the form of DMA, arsenite, MMA or TMAO (tromethylarsine oxide)[12,13] The rats developed bladder cancer when exposed to arsenic concentration levels ranging from 17 to 180 ppm in various forms. In other studies, DMA was shown to be a complete carcinogen at levels of 50 to 200 ppm. The high arsenic exposure levels compared to those expected in human exposure, however, makes it difficult to directly apply these results to environmental exposures.

Methylated forms of arsenic may be more genotoxic than the inorganic forms because they may be able to form adducts with DNA bases more easily than the inorganic species. At

physiological pH, DNA is negatively charged from its phosphate groups. As mentioned above, in the methylated forms of arsenic, MMA and DMA, the electronegative hydroxyl groups of the inorganic species are replaced by uncharged methyl groups. This decreases the electrostatic repulsion between the arsenic species and the target DNA, increasing the efficiency of adduct formation.

Methylated forms of arsenic can also produce oxidative stress by creating reactive oxygen species (ROS) that attack DNA. In this model, reductive metabolism of DMA(V) produces dimethylarsine. This species reacts with molecular oxygen to produce a number of ROS which can cause DNA single strand breaks[14]. Bladder cancer results from arsenic exposure because this organ contains relatively high concentration of DMA and MMA in the lumen.
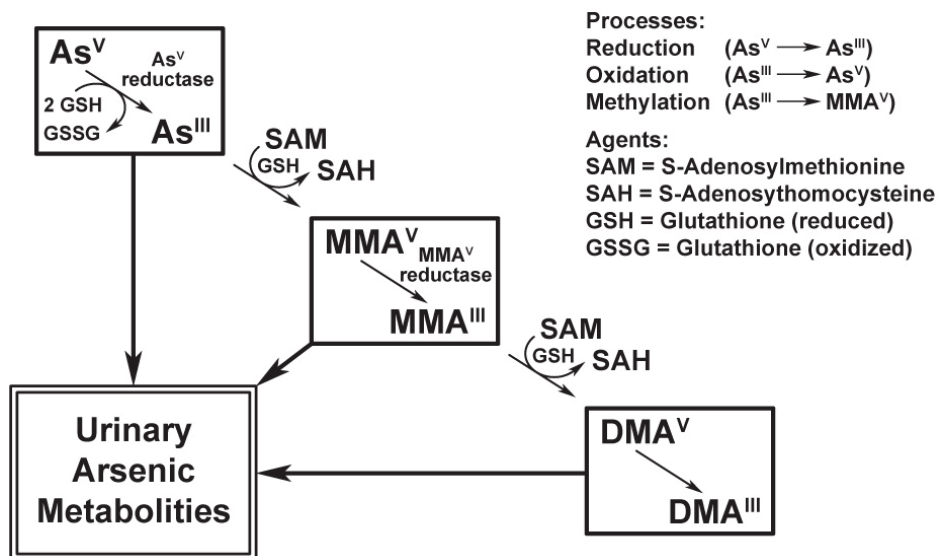
Figure A-1. Simplified scheme of arsenic metabolism in mammals. Pentavalent arsenic is reduced by glutathione. Methyl groups are supplied by SAM to form monomethylarsonic acid (MMA^V), monomethylarsonous acid (MMA^{III}), dimethylarsonic acid (DMA^V) and dimethylarsonous acid (DMA^{III}). (adapted from Kitchen[6]).

Detoxification Reactions and the Carcinogenesis of Arsenic and Arylamines

Cytochrome P450 enzymes

Arsenicals and the components of cigarette smoke can participate in a large number of metabolic pathways. Like other xenobiotics, the processes which detoxify them involve a number of reactions which may convert them to chemical forms that are more reactive than the ingested forms. This bioactivation ultimately makes them more transportable in blood and urine

and facilitates elimination from the body. However, these activated products may attack a variety of cellular components and lead to both toxic and carcinogenetic effects.

Cytochrome P-450 enzymes catalyze oxidation and hydroxylation reactions of xenobiotics. These reactions are Phase I metabolic reactions in which a normally lipophilic, uncharged xenobiotic species is converted to a form that is readily bound to a larger more hydrophilic molecule in a subsequent Phase II conjugation reaction. The resulting complex can then be more readily excreted by the body; thus, the reactions are detoxifying.

Cytochrome P-450 activity is important to a large number of detoxification reactions and is induced by a number of substrates. These substrates bind to protein receptors or transcription factors that translocate to the nucleus, bind to DNA of the appropriate P-450 gene and induce expression of the P-450 enzyme. P-450 enzymes contain a heme group that binds to oxygen, which is involved in oxidation or hydroxylation of the substrate. A cysteine residue holds the heme molecule in position for the reaction. The binding of arsenic to the sulfyhydryl group in the cysteine residue might alter the function of the P-450 enzyme.

P-450 is also involved in bioactivation of carcinogens in cigarette smoke. Hydroxylation of benzo[a]pyrene forms the benzo[a]pyrene-7,8-epoxide. Addition of H_2O converts the epoxide to a proximate carcinogen, benzo[a]pyrene-7,8-dithiol, which can be converted to benzo[a]pyrene-7,8-diol-9,10-epoxide by further epoxidation. This last species is an ultimate carcinogen that is very reactive with DNA. In the liver, hepatic cytochrome P4501A2 (CYP1A2) activates aromatic amines via N-hydroxylation.

Glucuronidation, acetylation, and hydroxylation of arylamines

Glucuronidation is a Phase II metabolic reaction in which a Phase I product is conjugated with uridine diphosphoglucuronic acid (UDP-GA). This reaction serves to render the Phase I metabolite highly polar and hydrophilic, thereby making it more readily excreted from the body in urine. In some cases, Phase I and Phase II reactions as described below can be bioactivating and produce metabolites that are more toxic than their parents.

Arylamines and N-hydroxyarylamines, components of dyes and cigarette smoke, form N-glucuronides by attaching to the nitrogen of the uridine residue of UDP-GA. This reaction may enhance bladder cancer by aiding transport of the arylamines from the liver to the bladder. There the conjugate may undergo acid hydrolysis and release the carcinogen.

Badawi et al.[15] noted that DNA adduct formation in bladder cells is directly or indirectly influenced by reactions involving several enzymes including the aromatic amine acetyltransferases NAT1, NAT2 and sulfotransferase. These enzymes catalyze bioactivation and detoxification reactions (Figure A-2). The N-hydroxy-arylamine metabolites enter into circulation, are filtered to the bladder lumen and then are readsorbed by the bladder epithelium. NAT1, an acetyl transferase in the bladder reacts (via O-acetylation) with the metabolites to form even more reactive electrophilic N-acetoxy-derivatives. These covalently bind to urothelial DNA and form a C-8 substituted deoxyguanosine derivative. Formation of this DNA adduct is regarded by some as the initiating step in a multistep model for carcinogenesis[15]. NAT2, another NAT protein, provides a competing detoxification pathway in the liver. It forms relatively nonreactive products of arylamines by N-acetylation.
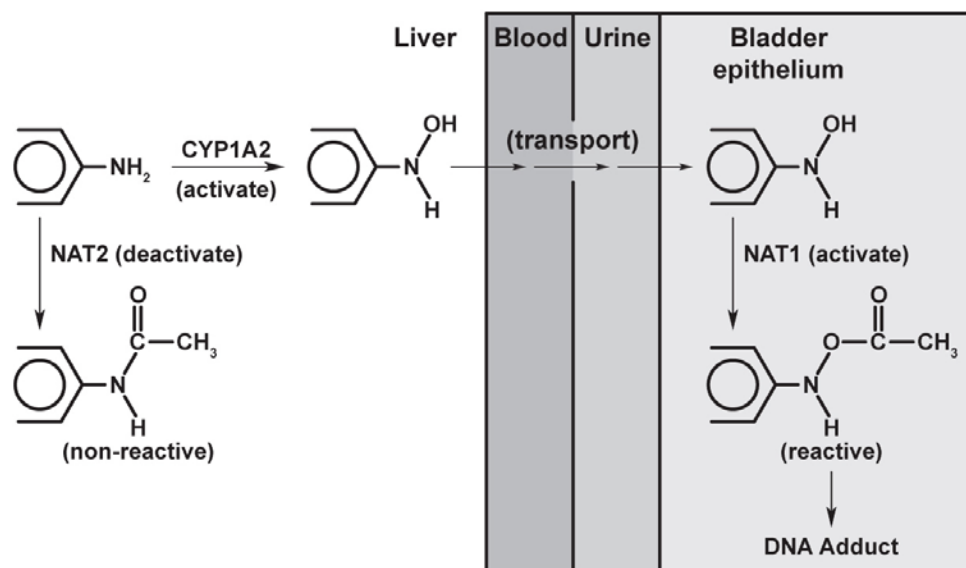
Figure A-2. Arylamine metabolic pathways for bladder carcinogenesis. Includes 1) activation via N-hydroxylation by Cytochrome P450 enzyme CYP1A2, transport to the bladder and further activation by NAT-1 via O-acetylation; and 2) deactivation by NAT-2 via N-acetylation. (adapted from Taylor et al.[19]).

Glutathione conjugation

Glutathione (GSH) is a tripeptide that is involved in many detoxification reations. It contains a strongly nucleophilic thiol (sulfydryl) group in the central cysteine residue that is the site of the conjugation reaction. Detoxification of xenobiotics proceeds by a number of steps catalyzed by several enzymes. The first reaction involves the glutathione s-transferase enzyme (GST) which catalyzes the addition of potentially damaging electrophilic compounds to the thiol. This enzyme is important to the metabolism of GSH and is critical in maintaining the redox state of cells. GSH may be particularly important in detoxification of arylamines and is involved in arsenic methylation as described above.

The nucleophilic thiol group in GSH would be expected to readily bind to arsenite, thereby decreasing the availability of GSH to participate in other detoxification reactions. Depletion of GSH by high doses of strongly binding electrophiles is known to cause a variety of toxic effects including liver damage and death[16]. Hopenhayn-Rich et al. [17] review epidemiological and experimental studies that suggest that depletion of GSH associated with high levels of arsenic exposure might cause oxidative damage leading to reproductive toxicity. In addition, As(III) species that are intermediate products of methylation reactions might be toxic. Both arsenite and methylarsenite species might inhibit the activity of glutathionite reductase. Thus it is possible that decreased levels of GSH activity due to arsenic exposure

could decrease the efficiency of detoxification reaction for arylamines, thereby increasing the risk of bladder cancer.

Genetic Effects

p53 gene

Mutations of the p53 gene are the most common genetic defect associated with human bladder cancer[18]. The tumor-suppressor gene is activated in response to stress such as DNA damage or exposure to certain cytokines. As the p53 protein is accumulated in the nucleus, it acts to terminate cell-cycle development or promotes apoptosis through a variety of mechanisms. p53 acts a transcription factor for a number of enzymes on pathways leading to cell arrest and apoptosis. These include the p21/WAF1/Cip1 pathway, which inhibits activity of cyclin-dependent kinase complexes, prevents phosphorylation of Rb proteins and ultimately blocks entry of cells in the S phase of the cell cycle; and Bax, which promotes cell apoptosis. p53 also regulates angiogenesis and may play a role in tumor progression through its activity as a transcription factor for a number of growth factors (VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor, and thrombospondin.) p53 also regulates transcription of MDM2, another nuclear protein. MDM2 in turn, controls the activity of p53 by binding to it and inhibiting its biologic function, and by targeting it for proteolytic destruction.

Studies of p53 protein levels in response to exposures to arsenite have yielded inconsistent results. p53 levels decreased and MDM2 levels increased in some cell lines exposed to arsenic but levels pf p53 increased in other cell lines reviewed by Kitchin[6]. Examination of skin cancers from patients in Taiwan and Australia also produced inconsistent results with the prevalence of p53 mutations ranging from 0 – 39% for different cell types. Kitchin[6] concluded that the evidence for the role of the p53 gene in arsenic carcinogenesis is not as strong as exists for other mechanisms.

NAT genes

Individuals with genetic polymorphisms, which lead to impaired detoxification reactions or DNA repair ability, may be at increased risk to bladder cancer associated with smoking or arsenic exposure. Molecular epidemiological and experimental studies provide evidence that people with certain polymorphisms of the *NAT* genes that express aromatic amine acetyltransferases have increased susceptibility for bladder cancer. Specifically, polymorphisms which lead to reduced expression of NAT2 (slow acetylators or *NAT2- slow*) and/or increased activity of NAT1 (*NAT1*10*), might increase the risk for bladder cancer[15,19].

NAT2 polymorphism has been associated with bladder cancer risk from occupational exposure to arylamines with single amine groups (β-naphthylamine) and from unfiltered "black tobacco smoke"[19,20]. In a molecular epidemiological study, Taylor et al.[19] found that bladder cancer risk depended on both *NAT1* genotype and smoking history. A gene-environment interaction was suggested by the genotype-dependent slopes of dose-response curves for cancer risk with increased years of smoking. The slopes differed for populations that were normal, heterozygous and homozygous for the *NAT*10* allele.

The same study showed that the *NAT2* genotype did not influence cancer risk either alone or in conjunction with smoking behavior. However, those individuals with both polymorphisms

(*NAT-2 slow* and *NAT1*10*) showed particularly high risk (OR = 6.3; 95%CI: 2.0 – 20.3) compared to non-smokers with neither polymorphism. Taylor suggests the following biological mechanism for that observation: people with the rapid *NAT2* phenotype may detoxify most of the arylamines in the liver, leaving little carcinogen to be activated by NAT1 in the bladder. Consequently, *NAT1* polymorphism is important only for people with the slow *NAT-2* genotype because more of the activated arylamine reaches the bladder.

Su et al.[21] (1998; cited in the NRC report[5]) examined a population in the blackfoot-disease endemic area of SW Taiwan to determine if the *NAT2** polymorphism was related to increased bladder cancer risk among arsenic-exposed individuals. An association was not found there, however, one was found in an area of Taiwan without increased arsenic in drinking water. This finding does not rule out an arsenic-smoking interaction related to the *NAT* genes. The molecular epidemiological studies of Taylor et al.[19] discussed above, suggest that the *NAT1*10* polymorphism may exert more direct influence on bladder cancer risk than the *NAT2** gene. Thus, a study of *NAT1* and *NAT2* polymorphism in blackfoot endemic areas might be useful. Laboratory studies of the interaction between arsenic and NAT1 during N-acetylation and O-acetylation of arylamines present in tobacco smoke might also be useful.

GST genes

Rebbeck[22] reviewed molecular epidemiological data that suggests that mutations in the *GSTM1* and *GSTT1* members of the glutathione-s-transferase supergene family increase susceptibility to bladder cancer and other kinds of cancers. The evidence for the link is particularly strong and is based on concordant results from 6 different studies. The mutations involve deletion in the genes that lead to decreased GST enzyme activity. As discussed above, GST is a crucial enzyme in arylamine detoxification reactions. Homozygous *GSTM1* and *GSTT1* deletions are fairly common in most populations reviewed by Rebbeck. He suggests that although the magnitude of the crude cancer risks associated with the mutations are small (OR <2), interactions with smoking increases the risks (OR = 3-5).

Chiou et al.[23] (1997, cited in the NRC report) studied arsenic methylation capacity and *GST* polymorphism in populations in northeastern Taiwan that are exposed to high concentrations of arsenic. He found that subjects with the null genotype of *GSTM1* had a slightly increased percentage of inorganic arsenic in their urine and that subjects with the null genotype of *GSTT1* had an increased percentage of DMA in their urine. This study, however, did not demonstrate a relationship between these polymorphisms and susceptibility to arsenic carcinogenesis.

It has been established that glutathione will directly reduce pentavalent arsenate species, however, evidence for separate arsenate reductases has also been accumulating. The presence of two enzymes, arsenate reductase and MMA^V reductase has been indicated. Studies of MMA^V reductase in human tissue by Zakharyan et al.[24] (2001, cited in the NRC report[5]) suggested that the human MMA^V reductase is identical to one of the GST enzymes (glutathione-s-transferase omega class1-1 or hGSTO 1-1). It has been suggested that polymorphisms in the *GST* genes might affect both detoxification mechanisms and arsenic metabolism[5].

The key role that GSH activity plays in these reactions means that individuals with reduced ability to metabolize GSH may be more susceptible to the effects of arsenic exposure and cigarette smoking. The 3-way interactions among arylamines, GSH and arsenite could either

enhance or partially mitigate any adverse effects of any two species alone in the following ways. Arsenic–induced decreased availability of GSH may inhibit detoxification of arylamines in cigarette smoke. However, the decreased levels of GSH may also reduce the extent of As-methylation, thereby reducing the concentration of methylated arsenicals that can act as either cancer promoters or complete carcinogens. This suggests that a study of the prevalence of the *GSTM1* mutations, *GSTT1* mutations and bladder cancer among smokers in areas with high arsenic exposures might be useful. In addition, the relationship between MMA^V reductase and human glutathione-s-transferase hGSTO 1-1 discussed previously, suggests that studies of polymorphisms of that gene might be worthwhile.

Conclusion: Possible Modes of Interaction between Arsenic and Smoking in Potentiating Bladder Cancer

It is likely that arsenic carcinogenesis results from a combination of processes including chromosomal damage, oxidative stress, and augmentation of growth factors. Arsenic can act as a cancer initiator, promoter or complete carcinogen. Large numbers of studies have been carried out to establish the roles of arsenic and arylamine in bladder carcinogenesis. However, this literature review has failed to identify any studies that definitively demonstrate a synergistic interaction between arsenic exposure and smoking leading to increased incidence or prevalence of bladder cancer. The large number of potential carcinogenetic mechanisms for arsenic and arylamines make it possible that synergistic effects for bladder cancer exist between arsenic and smoking. The failure to definitively identify an interaction between them may be in part due to sheer number of possible reaction paths that involve arsenic and arylamines.

The information examined in this literature review suggests that arsenic could interfere with detoxification mechanisms for arylamines. Candidates include N-acetylation of arylamines by NAT2 in the liver, O-acetylation of N-hydroxy arylamine metabolites in the bladder lumen, and the activity of glutathione s-transferase. Verification of these hypotheses might be made through experimental studies or molecular epidemiological studies of gene-gene-environment interactions in smoking and non-smoking populations exposed to high arsenic concentrations. Specific recommendations include: 1) a study of *NAT1* and *NAT2* polymorphism among smokers in blackfoot endemic areas; 2) laboratory studies of the interaction between As and NAT1 during N-acetylation and O-acetylation of arylamines present in tobacco smoke; and 3) a study of the prevalence of the *GSTM1* mutations, *GSTT1* mutations and bladder cancer among smokers in areas with high arsenic exposures. In addition, the relationship between MMA^V reductase and human glutathione-s-transferase hGSTO 1-1 suggests that studies of polymorphisms of the gene expressing that enzyme might be worthwhile.

Other synergistic interactions may exist that do not involve the mechanisms summarized in this study. This literature review is not comprehensive with respect to toxic chemical components of cigarette; it was limited primarily to the arylamines. Cigarette smoke contains a large number of chemicals that have not been identified as either initiating agents or promoters but could act as co-carcinogens for arsenic. Conversely, arsenicals could act as co-carcinogens for carcinogens in cigarette smoke.

References

1. Gerhke S, Kalbe T, Staehler G. Bladder cancers a model tumor of chemically induced carcinogenesis: A historical review. Aktuelle Urologie 1996;27(4): 218-220.

2. Smith AH, Hopenhayn-Rich C, Bates MN, Goeden HM, Hertz-Picciotto I, Duggan HM, Wood. R., Kosnett MJ, Smith MT. Cancer risks from arsenic in drinking water. Environmental Health Perspectives 1992;97:259-267.

3. Bates MN, Smith AH, Hopenhayn-Rich C. Arsenic ingestion and internal cancers: a review. American Journal of Epidemiology 1992;135(5):462-476.

4. Siemiatycki J, Dewar R, Nadon L, Gerin N. Occupational risk-factors for bladder cancer: Results from a case-control study in Montreal, Quebec, Canada. American Journal Epidemiology 1994;140(12):1061-1080.

5. National Research Council. Arsenic in Drinking Water: 2001 Update. Washington, DC 20055: National Academy Press, 2001.

6. Kitchin KT. Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites. Toxicology and Applied Pharmacology 2001;172:249-261.

7. Clavel J, Cordier S, Boccon-Gibod L, Hemon D. Tobacco and bladder cancer in males: increased risk for inhalers and smokers of black tobacco. International Journal Cancer 1989;44:605-610.

8. Kantor A, Hartge P, Hoover RN, Fraumeni JFJ. Epidemiological characteristics of squamous cell carcinoma andadenocarcinoma of the bladder. Cancer Research 1988;48:3853-3855.

9. Stern MC, Umbach DM, van Gils CH, Lunn RM, Taylor JA. DNA repair gene XRCC1 Polymorphisms, smoking, and bladder cancer risk. Cancer Epidemiology, Biomarkers & Prevention 2001;10:125-131.

10. National Research Council. Arsenic in Drinking Water. Washington, DC 20055: National Academy Press, 1999.

11. Moss M. Human carcinogenicity by arsenic. Environmental Geochemistry and Health 1992;14:49-54.

12. Yamamoto S, Konishi Y, Matsuda T, Murai T, Shibata M, et al. Cancer induction by and organic arsenic compound, dimethylarsinic acid (cacodylic acid), in F344/DuCrj rats after pretreatment with five carcinogens. Cancer Research 1995;55:1271-1275.

13. Wanibuchi H, Yamamoto S, Chen H, Yoshida K, Endo G, et al. Promoting effects of dimethylarsinic acid of N-butyl-N-(4-hydroxybutyl)nitrosamine-induced urinary bladder carcinogenesis in rats. Carcinogenesis 1996;17:2435-2439.

14. Kenyon EM, Hughes MF. A concise review of the toxicology and carcinogenicity of dimethylarsinic acid. Toxicology and Applied Pharmacology 2001;160: 227-236.

15. Badawi AF, Hirvonen A, Bell DA, Lang NP, Kadlubar FF. Role of aromatic amine acetyltransferases, NAT1 and NAT2, in carcinogen-DNA adduct formation in human urinary bladder. Cancer Research 1995;55:5230-5237.

16. Stine KE, Brown TM. Principles of Toxicology. New York: Lewis Publishers, 1996.

17. Hopenhayn-Rich C, Hertz-Picciotto I, Browning S, Ferreccio C, Peralta C. Reproductive and developmental effects associated with chronic arsenic exposure. In: Chappell WR, Abernathy CO, Calderon RL, eds. Arsenic Exposure and Health Effects Elsevier Sciences B.V., 1999;151-164.

18. Slaton JW, Benedict WF, Dinney CPN. In situ bladder cancer: mechanism of action, prognostic value, and target for therapy. Urology 2001;57:852-859.

19. Taylor JA, Umbach DM, Stephens E, Castranio T, Paulson D, Robertson C, Mohler JL, Bell BA. The role of N-acetylation polymorphisms in smoking-associated bladder cancer: evidence of a gene-gene-exposure three-way interaction. Cancer Research 1998;58:3603-3610.

20. Vineis P. Epidemiology of cancer from exposure to arylamines. Environmental Health Perspectives 1994;102:7-10.

21. Su JH, Gou YL LM, Huang JD, Cheng Y, Christiani DC. The NAT2* slow acetylator genotype is associated with bladder cancer in Taiwanese but not in the Black Foot Disease endemic area population. Pharmacogenetics 1998;8:187-190.

22. Rebbeck TR. Molecular epidemiology of the human glutathione S- transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiology, Biomarkers & Prevention 1997(6):733-743.

23. Chiou HY, Hsueh YM, Hsieh LL, Hsu LI, Hsu YH, et al. Arsenic methylation capacity, body retention and null genotypes of glutathione S-transferase M1 and T1 among current arsenic-exposed residents in Taiwan. Mutation Research 1997;386:197-207.

24. Zakharyan RA, Sampayo-Reyes A, Healy SM, Tsaprailis G, Board PG, et al. Human monomethylarsonic acid (MMA(V)) reductase is a member of the glutathione-S-tranferase superfamily. Chemical Research in Toxicology 2001;14:1051-1057.

Attachment B

Socioeconomic Indicators of Smoking Prevalence in New Mexican White Males:

An Analysis of Data from the
2000 New Mexico Behavioral Risk Factor Surveillance System

Abstract

This appendix describes the relationship between smoking prevalence and a number of socioeconomic factors in New Mexico populations sampled in 2000. Of particular interest is the association between age, race, ethnicity, urbanization and smoking prevalence among white males ages 18 –75+ years old. The Crude Prevalence Risk Ratio (PRR) for the relationship between the main exposure variable (ethnicity of white males) and the disease (lifetime smoking of at least 100 cigarettes is 0.84 (95% CI: 0.75 – 0.95. A logistic regression model included the main exposure variable (ethnicity) and the confounders age, and education. The adjusted odds of smoking among Hispanic White males is about 60% that of the odds among non-Hispanic White males (OR = 0.6; 95% CI: 0.40 - 0.8). The adjusted odds of smoking among older (> 44 yr) white males is 2.7 times that of younger men (95% CI: 2.1 – 3.6). Level of education is a protective factor for smoking (adjusted OR = 0.5; 95% CI: 0.3 - 0.6).

Introduction

Incidence and mortality rates of bladder cancer have been related to a large number of lifestyle, occupational, and environmental factors. Rates are highest for non-Hispanic white males and are related strongly to smoking. Any study of the association between bladder cancer and arsenic must take into account the possibility of confounding relationship between bladder cancer and smoking status. Data describing the relationship between smoking prevalence and arsenic exposure were not available for use in the ecological study described in this report. A more indirect method using US census data and the Behavioral Risk Factor Surveillance System (BRFSS) database[1] was used to estimate this relationship. The BRFSS database provides data on a number of behavioral risk factors including smoking as well as socioeconomic factors such as race, ethnicity, urbanization, income and education. These same socioeconomic factors are described in the US Census for the exposed and unexposed county populations described in the current study of arsenic exposure and bladder cancer. It was assumed that an indirect measure of differences in smoking prevalence between populations in exposed and unexposed counties could obtained in 3 steps: 1) determine relationship between the socioeconomic factors and smoking prevalence in the BRFSS database for white males in New Mexico and 2) determine the difference in demographic characteristics between in exposed and unexposed populations and 3) use the relationship obtained in step 1 and the difference in step 2 to qualitatively examine the relationship between smoking prevalence and exposure to arsenic. Because bladder cancer incidence and mortality are strongly associated with age and ethnicity, the relationships between these variables and smoking prevalence were of primary concern.

Methods

Data describing smoking histories and demographic information were obtained from the New Mexico Department of Health Behavioral Risk Factor Surveillance System (BRFSS) Survey database for 2000. The BRFSS is a telephone survey system coordinated by the Center for Disease Control (CDC) used to collect information about health-related behaviors in 50 states and 3 territories on a monthly basis. In 2000 approximately 3200 New Mexicans were surveyed in a 3-stage Mitofsky-Waksburg random digital dialing sample design.

Figure B-1 contains a flow chart describing how the sample containing 1054 white males was derived for this study from the 2000 NM BRFSS dataset. The data were cleaned by the assigning missing value indicator to the refusals and "I don't know responses." Refusals are not included in the analysis because the refusal rate was very low. Table B-1a describes the original 2000 NM BRFSS variables retained for the dataset and used to further subset the data and create the final variables. The final variables created for the logistic regression analysis are shown in Table B-1b.

The 2000 BRFSS survey for New Mexico contained several questions about current and past smoking history. In this study, a smoker is defined to include a respondent to the 2000 NM BRFSS data set that smoked at least 100 cigarettes in his entire life. People who have not smoked at least 5 packs in their lives are considered non-smokers regardless of their current smoking status. This measure of smoking was chosen because it required the least number of assumptions and it produced the largest data set. It is probably less subject to recall bias or poor-self reports than alternative measures. The major disadvantage is that the variable provides little information about heavy smoking or cumulative smoking (pack-years); these measures may be more directly related to the incidence of bladder cancer. Other measures of smoking behavior based on the number of days smoking or number of cigarettes smoked could be used in follow-up studies. In addition to the limitations described above, other important limitations of the dataset include the self-reported smoking status and the reliance on telephone interviews. The latter limitation is important because it weakens the validity of conclusions concerning socio-economic status.

Bivariate analysis and logistic regression were carried out on the data using the STATA7 program[2]. The main "exposure" variable of interest is ethnicity of white males (white-hispanic vs non-Hispanic white). The variable **whitemales** was created by selecting records with **sex** = male, **orace** = white and sorting on the value of the **hispanic** variable. Several covariates were constructed to evaluate potential effect modification and confounding by education, income, marital status, age and urbanization. Table B-2 describes the distribution of the sample population across levels of the covariates and the "exposure" and disease variables.

Results

Bivariate Analysis

Table B-3 shows the relationship between the main exposure variable (ethnicity of white males) and the disease (lifetime smoking of at least 100 cigarettes). The Crude Prevalence Risk Ratio (PRR) is 0.84 (95% CI: 0.75 – 0.95). This suggests that if confounding and interactions are not significant then the prevalence of smoking among White Hispanics is less than among non-Hispanic whites in the sample population. This result, however, cannot be extrapolated to the general population without use of appropriate sample weights. Unfortunately, because of the procedure used to clean the data set, the sample weights were not retained and this correction cannot be made.

Table B-4a shows the crude relationship between age and smoking history for 10-year interval age levels (18-24 year old interval is a 7 year interval). Relative risks are calculated using the youngest age group as the referent. The age groups older than 45 years all show statistically significant elevated smoking prevalences compared to the youngest group. The analysis of the relation between smoking history and ethnicity of white males adjusted for age (Table B-4b) suggests that among white males ages 45-64, white Hispanics smoke more than non-Hispanic whites. However, the relationship is not statistically significant at the 0.05 level.

Table B-5a shows the crude relationship between smoking history and educational level. The 2 x 4 table shows that there is relationship between the disease and the education; smoking prevalence is highest among the reference population (less than high school education)at the 95% confidence level. The stratified analysis of smoking history and ethnicity shows that the relationship between the exposure and disease status is not modified by education. The use of the Mantel Haenszel (MH) adjustment is appropriate ($P_{MH} = 0.469$), but the degree of confounding is small (about 10%), therefore a dichotomous variable for education was used in the final model.

Other bivariate analyses were carried out. The bivariate relationships between smoking history vs. marital status (defined as married vs single), and smoking history vs. income level were not statistically significant. The bivariate relationship between the disease and health district (urbanization) is not statistically significant, using Bernalillo County as the referent. However, urbanization may be a modifier of the relationship between the smoking history and the ethnicity. The PRR for Bernalillo is 0.73 (95% CI: 0.57, 0.94), whereas, none of the other health districts show PRRs significantly less than 1.0 at the 95% CI.

Logistic Regression

The results of the bivariate analyses suggested that the initial logistic regression model should include all of the covariates and interactions between ethnicity and education, between ethnicity and marital status and between ethnicity and health district. Several different logistic regression models were constructed using different codings of the covariates with and without interactions. The final model includes the main exposure variable (ethnicity) and the confounders age, and education (Table B-6). The adjusted

odds ratio (OR) from the logistic regression is 0.6 (95% CI: 0.40 - 0.8) using non-Hispanic Whites as the reference group. This means that the odds of smoking among Hispanic White males is about 60% that of the odds among non-Hispanic White males. The adjusted odds of smoking among older (> 44 yr) white males is 2.7 times that of younger men (95% CI: 2.1 – 3.6). Level of education is a protective factor for smoking (adjusted OR = 0.5; 95% CI: 0.3 - 0.6).

Table B-7 describes the results of statistical tests (maximum likelihood ratio) of the significance of the other variables and interactions (Full model). The P-value (0.617) indicates that the null hypothesis (the coefficients of the variables urban, marital, educations and the coefficients of the interaction terms are all equal to zero) could not be rejected. The table also compares describes the preferred model to the reduced model containing only the main exposure variable. The low P-value indicates that the null hypothesis that the coefficients of the age and education variables are equal to zero was rejected with high confidence.

Conclusions

All of the models constructed in this study show that the odds of smoking (as defined by the lifetime smoking of 5 packs or more) is lower among White Hispanic males than among non-Hispanic White males. The adjusted odds ratio for smoking is 0.6 (95% CI: 0.40 - 0.8) using non-Hispanic Whites as the referent group. This means that the odds of smoking among Hispanic White males is about 60% that of the odds among non-Hispanic White males.

The covariate age and education are all also associated with smoking at the 0.05% significance level. The odds of smoking among older (> 44 yr) white males is 2.7 times that of younger men (95% C.I.: 2.1 – 3.6). Education level is a protective factor for smoking (OR 0.5; 95%CI: 0.3-0.6). Neither the variables for income, urbanization and marital status nor the interactions between the variables and ethnicity were significantly associated with smoking in any of the models.

The results provide a good baseline study for the relationship between demographic factors and smoking behaviors that may be relevant to bladder cancer incidence. As mention above, however, the major limitation in this study is that the smoking variable chosen provides little information about heavy smoking or cumulative smoking (pack-years); these measures may be more directly related to the incidence of bladder cancer. It is possible that a different variable for smoking would provide different results. There is no single correct measure of smoking behavior. Leffondre et al.[3] demonstrate that various aspects of smoking history exert distinct effects on disease. The exact distinction between current smokers and ex-smokers and the inclusion of never-smokers in analyses all affect the estimated effect of smoking as either a main exposure or a confounder.
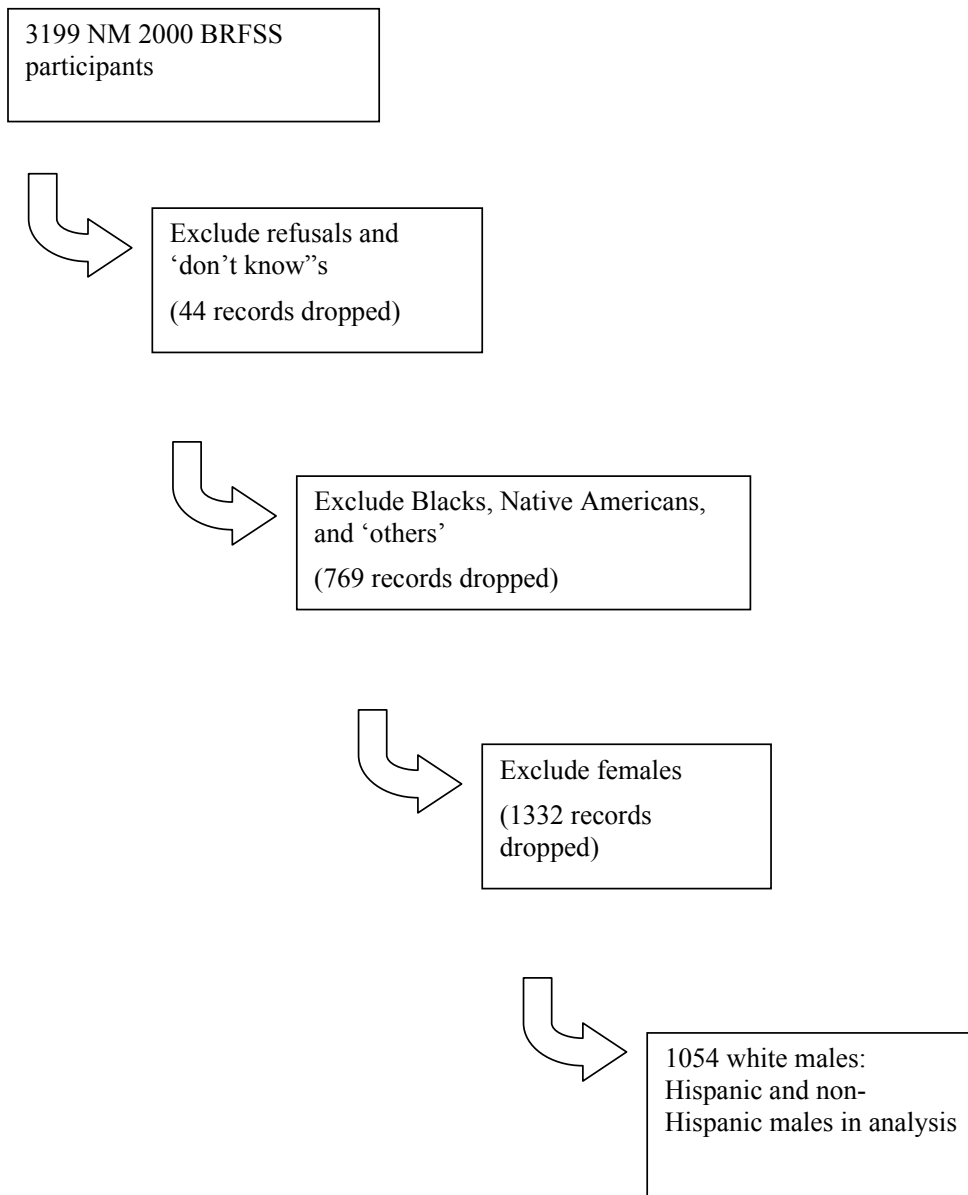
Figure B-1. Flow Chart for Derivation of Dataset for Smoking History-Ethnicity Analysis for White Males.

Table B-1a. BRFSS Variables Retained for Analysis.

smoke100   smoked at least 100 cigarettes

age   reported age in years

orace   original reported race

hispanic   hispanic origin

marital   marital status

educa   education level

income2   income level

sex   sex

_ageg10yr   Ten-Year Age Group Variable

racenm   Race/ethnicity

educnm   Education

employnm   Employment

incomenm   Income

hdistr   Health district

Table B-1b. Computed Variables Used in Logistic Regression Analysis.

Variable	Levels	
smoke_ever3	0.	<100 cigarettes in lifetime
	1.	≥ 100 cigarettes in lifetime
Whitemales (ethnicity)	0.	Non-Hispanic white males
	1.	Hispanic white males
Agedi (age)	0.	18 - 44 yr old
	1.	45 - >75 yr old
Educatdi (education)	0.	High school education or less
	1.	College education or more
Incomedi (income)	0.	Household income < $20,000/yr
	1.	Household income ≥ $20,000/yr
Urban (urbanization)	0.	Reside in Bernalillo county
	1.	Reside outside of Bernalillo county
Marital2 (marital status)	0.	Married or member of unmarried couple
	1.	Single = separated or divorced or widowed or never been married

Table B-2. Demographic Description of 1054 Participants Included in Final Smoking History-Ethnicity Dataset of White Males (whitemales).

Variable	N	%	Cum.%
Ten-Year Age Group Variable			
18-24	83	7.9	7.9
25-34	155	14.7	22.6
35-44	232	22.0	44.6
45-54	236	22.4	67
55-64	151	14.3	81.3
65-74	115	10.9	92.2
75 and over	79	7.5	99.7
missing	3	0.3	100
Total	1054	100	
Ethnicity			
White non-hispanic	756	71.7	71.7
White hispanic	298	28.3	100
Total	1054	100	
Education			
less than High School	110	10.4	10.4
High School	281	26.7	37.1
some College	282	26.8	63.9
College graduate	381	36.2	100
Total	1054	100	
Income			
< $10,000	33	3.1	3.1
$10-19,999	143	13.6	16.7
$20-49,999	473	44.9	61.6
$50,000 or more	347	32.9	94.5
missing	58	5.5	100
Total	1054	100	

Table B-2. Demographic Description of 1054 Participants Included in Smoking History-Ethnicity Dataset of White Males (continued).

Variable	N	%	Cum.%
Marital Status			
married	692	65.7	65.7
single	361	34.3	99.9
missing	1	0.1	100
Total	1054	100	
NM Health			
District			
Bernalillo	319	30.3	30.3
NW	151	14.3	44.6
NE	197	18.7	63.3
SW	210	19.9	83.2.
SE	177	16.8	100
Total	1054	100	
Smoking History			
less than 100 cigs	413	39.2	39.2
more than 100 cigs	640	60.7	99.9
missing	1	0.1	100
Total	1054	100	

Table B-3. Crude Relationship Between Smoking History and Ethnicity.

	Disease (+) smoked at least 100 cigarette in lifetime	No Disease (-) Not smoked at least 100 cigarette in lifetime
white-hispanic **Exposed (+)**	160	138
white-nonhispanic **Not Exposed (-)**	480	275
Total	640	413

Number missing: 1

RR= 0.84 ,   95% C. I.   (0.75, 0.95),   P=0.003

Table B-4. Relations Among Smoking History, Ethnicity of White Males, and Age.

Variable: smoke_ever3

Description: smoking history; case = smoked at least 100 cigarette in lifetime

Variable: whitemale:

Description: ethnicity of white males; exposed = white-hispanic:

Variable: ageg10yr:

Description: age in approx 10 yr intervals

Table B-4a: Crude Relationship Between Smoking History and Age.

	Disease (+) Smoked at least 100 cigarette in lifetime	**Disease (-)** Not smoked at least 100 cigarette in lifetime	**Relative Risk**
18- 24 yr old Comparison group (-)	37	46	RR= 1.0
25-34 yr old Exposure group (+)	80	75	RR= 1.15, 95% C. I. (0.87, 1.54)
35-44 yr old Exposure group (+)	111	121	RR= 1.07, 95% C. I. (0.82, 1.41)
45-54 yr old Exposure group (+)	151	85	RR= 1.44, 95% C. I. (1.11, 1.86)
55-54 yr old Exposure group (+)	109	42	RR= 1.62, 95% C. I. (1.25, 2.10)
65-74 yr old Exposure group (+)	84	30	RR= 1.65, 95% C. I. (1.27, 2.15)
75 and older Exposure group (+)	65	14	RR= 1.85, 95% C. I. (1.42, 2.40)

Number missing: 4

65

**Table B-4. Relation between Smoking History, Ethnicity of White Males, and Age
(continued).**

Table B-4b. Relationship between Ethnicity, and Smoking Stratified by Age.

18- 24 yr old Group	RR = 0.46	95% C.I. (0.25, 0.85)
25-34 yr old Group	RR = 0.88	95% C.I. (0.63, 1.21)
35-44 yr old Group	RR = 0.82	95% C.I. (0.60, 1.13)
45-54 yr old Group	RR = 1.02	95% C.I. (0.82,1.27)
55-64 yr old Group	RR = 1.13	95% C.I. (0.92, 1.40)
65-74 yr old Group	RR = 0.92	95% C.I. (0.70, 1.20)
>75 yr old Group	RR = 0.83	95% C.I. (0.55, 1.27)
Crude PRR	RR = 0.84	95% C. I. (0.75, 0.95)
*MH-adjusted PRR=	RR = 0.90	95% C. I. (0.80, 1.01)

* Mantel Haenszel adjusted Relative Risk (RR); the test of (in)homogeneity was significant at P = 0.088;
therefore, the MH-adjusted RR is questionable.

Table B-5. Relations Among Smoking History, Ethnicity of White Males, and Education Level.

      Variable: smoke_ever3

      Description: smoking history; case = smoked at least 100 cigarette in lifetime

      Variable: whitemale:

      Description: ethnicity of white males; exposed = white-hispanic:

      Variable: educnm:

      Description: educational level

Table B-5a. Crude Relationship Between Smoking History and Education Level.

	Disease (+) Smoked at least 100 cigarette in lifetime	**Disease (-)** Not smoked at least 100 cigarette in lifetime	**Relative Risk**
Less than High school Comparison group (-)	81	29	RR= 1.0
High School or GED Exposure group (+)	184	97	RR= 0.89, 95% C. I. (0.77, 1.02)
Some College Exposure group (+)	174	108	RR= 0.84, 95% C. I. (0.72 , 0.97)
College graduate Exposure group (+)	201	179	RR= 0.71, 95% C. I.(0.62, 0.83)

      Number missing: 1

Table B-5b. Relationship between Exposure and Disease Stratified by Education.

Less than High school Group	RR= 0.78	95% C.I. (0.62, 0.98)
High School/GED Group	RR= 0.81	95% C.I. (0.68, 0.98)
Some College Group	RR= 0.63	95% C.I. (0.48, 0.84)
College graduate Group	RR= 0.83	95% C.I. (0.59, 1.15)
Crude PRR	RR = 0.84	95% C. I. (0.75, 0.95)
*MH-adjusted PRR	RR = 0.76	95% C. I. (0.67, 0.86)

Mantel Haenszel adjusted Relative Risk (RR); the test of (in)homogeneity not significant at P = 0.469; therefore, the MH-adjusted RR is appropriate.

Table B-6. Final Logistic Regression Model Describing Relationships Between Smoking Prevalence and Ethnicity, Age and Education.

Variable	N	Adjusted Odds Ratio for smoking	95% C.I.	p value
Hispanic	298	0.6	0.4 – 0.8	0.001
Age > 44	581	2.7	2.1 – 3.6	<0.001
College or more education	663	0.5	0.3 – 0.6	<0.001

Table B-7. Statistical Tests for Nested Logistic Regression Models.

Model	N	Variables	df	Log likelihood	LR (df)	p
Full (all interactions)	994	ethnicity x (age, income, education, urban, marital)	11	-616.214	5.354 (7)	0.617
Preferred model	994	Ethnicity, age, education	4	-621.568	NA	NA
Exposure only	1053	ethnicity	1	-700.888	79.32(3)	<0.001

References

1. Behavioral Risk Factor Surveillance System. Retrieved December 5, 2002, from Center for Disease Control: http://apps.nccd.cdc.gov/brfss/page.asp?cat=TU&yr=2000&state=NM#TU.

2. StataCorp. Stata Statistical Software: Release 7. College Station, TX: Stata Corporation, 2001.

3. Leffondre K, Abrahamowicz M, Siemiatycki J, Rachet B. Modeling smoking history: A comparison of different approaches. American Journal of Epidemiology 2002;156:813-823.

Distribution:

1	MS 0701	P. Davies, 06100
1	MS 0701	J. Merson, 06110
1	MS 0754	T. Hinkebein, 06118
1	MS 0754	P. Brady, 06118
1	MS 0754	R. Kottenstette, 6118
1	MS 0754	J. Krumhansl, 06118
1	MS 0754	S. Collins, 06118
1	MS 0754	T. Meyer, 06118
30	MS 0754	M. Siegel, 06118
2	MS 9018	Central Tech. Files, 8945-1
2	MS 0899	Tech Library, 9616